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Pesticide Background Statements

Volume I. Herbicides

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Herbicide Background Statements: General Information

1.0 Introduction

This section provides general information relevant to all of the Herbicide Background Statements. It primarily addresses those factors which are important to an understanding of the technical information presented in each background statement. General information concerning toxicity testing procedures, rating of relative toxicities, environmental fate and transport of pesticides, and assessment of hazards is presented as background for the discussion of specific material in the background statements for each herbicide. For convenience, a table of useful conversion factors and a glossary of terms and definitions have been included as Sections 5.0 and 6.0, respectively.

The individual Herbicide Background Statements have been compiled to provide a comprehensive review of the available information concerning the use, chemistry, toxicology, environmental fate, and comparative hazard of the herbicides in forest applications. It is not within the scope of the Herbicide Background Statements to critically evaluate and/or independently analyze the raw data from which conclusions were reached in the various studies and reports examined in preparing the background statements contained in this document. References to the published literature at the end of each background statement are provided for those individuals who wish to independently evaluate the toxicological data and environmental fate information that is presented in summary form. In many instances, secondary sources, such as review articles, handbooks, and company technical data sheets, were used. Wherever possible in these instances, the primary source was also indicated and referenced, although it may not have been examined.

1.1 Herbicide Use by the USDA Forest Service

Vegetation management programs are used by the USDA Forest Service for control of noxious and poisonous plants, wildlife habitat improvement, domestic range revegetation, maintenance of forest improvements, site preparation, conifer release, and thinning timber stands. A number of means are used for vegetation management, including fire, mechanical methods, manual methods, biological control, and herbicides. These means of control are often used in combination rather than alone, and they vary in effectiveness and utility with factors such as vegetation, topography, proximity of inhabited areas, and competing management objectives (U.S. Department of Agriculture 1978).

A wide variety of chemicals that show herbicidal activity are available to land managers. Most herbicides are originally produced as water insoluble crystalline acids or powders that are processed to

form more soluble compounds (e.g., salts) or to produce formulations (e.g, emulsions) that can be readily applied to, and taken up by, plants.

The quantities of herbicides applied depend upon the time of year, type of vegetation, specific management objectives, and the properties of the herbicide. Rates of application are generally stated in terms of pounds of acid equivalent (a.e.) or pounds of active ingredient (a.i.) applied per acre, depending on the specific chemical nature of the herbicide formulation and carrier (U.S. Department of Agriculture 1978).

1.2 Physical and Chemical Properties

A number of physical and chemical properties of the pesticide active ingredient, carrier, surfactant, or other adjuvants ultimately determine the behavior of the chemical in the environment. For instance, cationic (positively charged) pesticides tend to be readily adsorbed by colloidal soil particles and thus become immobilized. Anionic (negatively charged) and nonionic (neutral) pesticides are more mobile in the soil since they do not tend to adsorb to soil particles. Water solubility of pesticides also affects their mobility--chemicals with a low water solubility (i.e., less than 1 ppm) tend to be relatively immobile, while chemicals with a high water solubility tend to be more readily leached out of soils, particularly if they also do not bind tightly to soil. The vapor pressure of a pesticide affects its volatility and thus its ability to dissipate through the atmosphere. The degradability of a pesticide by sunlight or by chemical or biological activity determines its persistence in the environment. Finally, the biological properties of a pesticide, such as its mode of action and its biological storage (bioaccumulation) properties, determine its effect on nontarget organisms (Weber 1977).

Each of the individual Herbicide Background Statements briefly presents information on normal use patterns and application rates, particularly as used by the USDA Forest Service. In addition, a summary of available data on chemical and physical properties is provided, with emphasis on parameters such as solubility in water, solubility in organic solvents, and vapor pressure, all of which influence behavior in the environment.

2.0 Toxicity of Herbicides

A pesticide, by definition, is toxic to living organisms. Ideally, the toxic activity of a pesticide should be limited to its intended target organism, such as unwanted woody plants or weeds in the case of a herbicide. However, this limitation does not always occur. Sometimes nontarget plants, insects, or other animal species may be adversely affected by the pesticide. The potential hazard to humans and wildlife is obviously a major concern following any pesticide application.

For each Herbicide Background Statement, the toxicology data from the open literature is presented in appendices and summarized in the text by taxonomic grouping (invertebrates, fish, birds, and mammals). Mammalian toxicology data is further divided into acute, subchronic, chronic, and reproductive toxicity studies. Finally, available information on mutagenicity and carcinogenicity is presented.

The following discussions provide a brief overview of toxicity testing and comparative ratings of toxicity. More complete treatment of toxicology methods can be found in Guthrie and Perry (1980), Doull et al. (1980), Loomis (1978), or Hamburger et al. (1983).

2.1 Toxicity Testing

To determine the range and magnitude of potential toxic hazards associated with their use, pesticidal compounds are tested on a wide spectrum of organisms. These organisms include microorganisms, invertebrates such as insects and crustacea, fish, birds, and mammals. Tests are usually performed under controlled laboratory conditions. As such, the tests provide data on the inherent toxicity of the compounds. Although they simulate normal routes of exposure, they do not simulate natural environmental conditions. Mammals most often used are rodents, rabbits, and dogs. Farm animals such as chickens, sheep, pigs, dairy cows, and beef cattle, as well as fish, aquatic invertebrates, and wildfowl may also be used. Information from these tests regarding potential hazards can then be extrapolated to other species, including humans.

Toxicity tests in mammals fall into several categories. For all practical purposes, the duration of exposure determines whether a test is regarded as acute, subchronic, or chronic.

In an acute test conducted with terrestrial animals, a single dose or exposure is given over a short period, such as 24 hours, and the effects are observed over several days to several weeks. The most frequently determined indices of toxicity are the median lethal

dose (LD_{50}) and the median lethal concentration (LC_{50}), which are defined as the dose or concentration that are lethal to 50 percent of a group of treated animals. Lower numerical values for the LD_{50} or LC_{50} indicate greater toxicity than do higher values.

The acute toxicity of a compound may be based upon oral, inhalation, or dermal studies. In oral studies, the LD_{50} or LC_{50} is stated in terms of milligrams of compound administered per kilogram of body weight of the animal (mg/kg) or in terms of the parts per million (ppm) of chemical in the diet, respectively. For inhalation studies, median lethal concentrations are given in terms of parts per million for vapors and gases, or milligrams per cubic meter of air (mg/m^3) for dust or particulates. For dermal studies, test substances applied to the skin are given in terms of milligrams of compound per kilogram of body weight.

For fish and other aquatic organisms, the median lethal concentration (LC_{50}) is used as an index of acute toxicity. The LC_{50} refers to the concentration of chemical in water necessary to kill 50 percent of the aquatic test organisms during a specific exposure period. The LC_{50} is usually expressed as parts per million of chemical in water for 24, 48, 72, or 96 hours of exposure.

In addition to determining dose levels that are lethal, acute toxicity tests are also used to determine dose levels that produce eye irritation, dermal irritation and sensitization, and neurotoxic effects. Neurotoxicity testing is particularly important with organophosphate insecticides, since the nervous system is a target for these chemicals.

Subchronic studies are designed to determine adverse effects of regularly repeated doses or exposures over periods ranging from a few days to several months. Exposure may be by oral ingestion, dermal application, or inhalation. Death is usually not the endpoint. Manifestations of toxicity may include effects on the clinical health of the animal such as changes in food consumption or rate of growth, loss of weight, and behavioral changes. Signs of clinical pathology include changes in hematology, blood and serum chemistry, and urine chemistry. Animals are also examined to detect gross or histological pathologic changes. The intent is to identify target organs and the manifestations of toxicity and to define a dose level that produces a no-observable effect (i.e., a NOEL or No Observable Effect Level).

Chronic studies involve the exposure of the test animals to appropriate dosages of the pesticide for a major portion of the lifespan of the animal. The objective is to assess potential health hazards that might be associated with long-term, low-level exposure. Mice and rats have been the traditional test species for chronic exposure studies because of their relatively short lifespans and the

availability of extensive data on their biology in laboratory environments. Dogs are also used. Test procedures vary depending upon the toxicity endpoint being investigated.

In addition to detecting injury to various organs, tests employing sublethal levels of a particular compound are designed to detect the potential for carcinogenic, teratogenic, or adverse reproductive effects of chemicals as well as for mutagenic potential. Carcinogenicity studies are designed to determine the ability of a test substance to cause the induction and growth of malignant or benign tumors. Teratogenicity studies are conducted to detect fetal abnormalities, and reproductive studies are designed to detect adverse reproductive effects over more than one generation. Mutagenicity tests are performed to determine whether a chemical has the potential to cause an increase in the mutation rate of either (1) germ cells (eggs or sperm) or (2) somatic cells (body cells found in the various organs and tissues). Such mutations may be inherently undesirable and may also be indicative of the carcinogenic potential of the test substance.

Tests for carcinogenicity usually involve either lifetime studies or exposure of the organism to the test substance for a large portion of the organism's lifetime. In most instances, rats and mice, having relatively short lifespans, are the test organisms of choice, although dogs have been used. Statistically increased incidences of tumors (in test versus control animals) are indicative of carcinogenic potential.

Teratogenicity studies, which involve the treatment of pregnant animals, are conducted to identify agents that cause malformations (called terata) of the fetus. Although many chemicals have been shown to be teratogenic in laboratory animals, only a few compounds have been demonstrated to be teratogenic in man. Fetal malformations can also be caused by hereditary factors, as well as by such environmental factors as dietary deficiency, viral infections, hyperthermia, hormonal imbalance, and physiological stress (Loomis 1978).

Reproductive studies are performed to identify agents acting on the reproductive capacity of the animal in terms of parameters such as fertility and lactation performance. Reproductive studies also usually provide information on the effects of chemical substances on teratogenicity and, since they are often conducted through several generations, on mutagenicity. Endpoints of these tests are to determine 1) toxic effects on parents in terms of altered gonadal function, estrus cycles, mating behavior, conception rates, and early pregnancy; 2) toxic effects on fetal development in terms of

normality, teratogenic/mutagenic effects, and mortality in the uterus; and 3) toxic effects on the mother in terms of lactation performance and behavior towards the offspring (Loomis 1978).

Presently, there is no unequivocal evidence for the mutagenicity of any specific chemical in man, since mutagenicity tests are equivocal and difficult to interpret. Most mutagenicity tests are conducted on laboratory animals, microbial cells, and mammalian cells. They are designed to determine point mutations, gene mutations, chromosomal aberrations, or damage to DNA. Increases in the mutation rates of germ cells may lead to increased incidences of genetic disease in future generations; increases in the mutation rates in somatic cells may lead to increased incidences of diseases in the generation exposed to the chemical. A number of mutagenicity test systems are available and include the use of microbial or mammalian cells or whole animals and plants. Mutagenicity tests with submammalian systems, such as microorganisms (bacteria or yeast), plants, and insects offer the advantage of simplicity and the availability of large populations to increase the probability of occurrence for rare mutation events. The disadvantage of such submammalian systems is their lack of similarity to mammalian physiology and metabolism. This disadvantage has led to the development of systems in which mammalian metabolizing systems, such as liver homogenates, are added to a bacterial test system and development of cytogenetic methods in which mammalian cells are examined for evidence of chromosomal changes (Loomis 1978). It should be noted that a positive result in a mutagenicity test cannot be extrapolated directly to humans, but indicates only that a chemical can act as a mutagen and as such, may pose a potential hazard to humans.

Given the assumption that a mutagenic response may be indicative of the carcinogenic potential of a substance, simple microbial test methods for the detection of mutagens can be used to screen substances for potential carcinogenic activity. More than 100 test systems exist. One of the most widely used test system is the Ames Salmonella/ microsome in vitro test which uses mutant strains of Salmonella typhimurium that require the amino acid histidine in the culture medium to grow. In the presence of mutagenic substances, these mutant strains will revert to the original, or wild type, which are not histidine dependent (Loomis 1978).

2.2 Rating of Relative Toxicities

The comparative toxicities of pesticides vary over a wide range. Several schemes have been proposed to categorize them. For example, the median lethal dose of a chemical can be given a relative toxicity rating from "highly toxic" if the LD_{50} is less than 50 mg/kg to

"practically nontoxic" if the LD_{50} is greater than 5,000 mg/kg (Hodge and Sterner 1949; Radeleff 1964; and Melnikov 1971). A similar system was proposed for rating subchronic toxicities in which chemicals administered in the diet are placed into five toxicity classes according to the lowest dose eliciting a toxic response (Hill et al. 1975). This system is as follows: Class I, less than 41 ppm; Class II, 41 to 100 ppm; Class III, 201 to 1,000 ppm; Class IV, 1,001 to 5,000 ppm; and Class V, greater than 5,000 ppm. The U.S. Environmental Protection Agency (EPA) guidelines for categories of acute and subchronic toxicity used for precautionary labeling under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, are shown in Table 2-1. The toxicity categories range from I through IV depending upon either the median dose required to cause death or upon the severity of the reaction elicited by the chemical. All of the above ratings of relative toxicity may be referred to in the specific Herbicide Background Statements.

In terms of lethal oral acute doses in man, a similar scheme for rating toxicity has been devised (Table 2-2) and indicates the probable oral lethal dose of the different toxicity classes for an average adult human. Although such a toxicity rating system is only qualitative, it serves as a practical and useful guide by which to place toxicity categories in perspective.

Fish swimming in water containing toxicants are exposed simultaneously to the compounds by more than one route of exposure. Consequently, toxicity data for fish cannot be compared directly with toxicity data derived from studies in birds or mammals. Studies of the toxicity of pesticides to fish are conducted in laboratory environments with the fish swimming in water containing a given concentration of the pesticide formulation. These studies are either static bioassays, in which the system is not renewed during the course of the study, or flow-through bioassays, in which there is fresh test solution flowing through the exposure chambers. Data from laboratory studies must be interpreted with care when extrapolating to natural environments, since responses of fish to environmental pollutants are affected by many other environmental factors including temperature, water hardness, salinity, oxygen and carbon dioxide content, oxygen depletion, and pH (Clarke et al. 1970). In general, "cold-water" fish, such as trout and salmon, are often more sensitive to herbicides and other pollutants than the "warm-water" fish, such as carp and bass. Finally, it should be noted that laboratory aquarium studies may not always show sublethal toxic effects, such as impairment of chemical homing behavior in migratory fish which could reduce their ability to reach spawning grounds.

Clarke et al. (1970) proposed that toxic substances with an LC_{50} for fish of less than 1 ppm for a 96-hour exposure should be

Table 2-1

Categories of Acute Toxicity

Toxicity Category	Signal Word	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	Inhalation LC_{50} Dust or Mist (mg/liter)	Inhalation LC_{50} Gas or Vapor (ppm)	Eye Effect	Skin Irritation
I	Danger Poison	50 or less	200 or less	2 or less	200 or less	Irreversible corneal opacity at 7 days.	Severe irritation or damage at 72 hours.
II	Warning	50 through 500	200 through 2,000	2 through 20	200 through 2,000	Corneal opacity reversible within 7 days, or irritation persisting for 7 days.	Moderate irritation at 72 hours.
III	Caution	500 through 5,000	2,000 through 20,000	20 through 200	2,000 through 20,000	No corneal opacity, irritation reversible within 7 days.	Mild or slight irritation at 72 hours.
IV	Caution	5,000 or greater	20,000 or greater	200 or greater	20,000 or greater	No irritation.	No irritation at 72 hours.

Adapted from U.S. Environmental Protection Agency toxicology guidelines, summarized in Ashton 1982.

Table 2-2

Toxicity Rating Chart for Acute Oral Doses in Man

Toxicity Rating	Classification	LD_{50} (mg/kg)	Probable Lethal Oral Dose for Average Adult Human
1	Super toxic	Less than 5	A taste (less than 7 drops)
2	Extremely toxic	5 to 49	7 drops to 1 teaspoonful
3	Very toxic	50 to 499	1 teaspoonful to 1 ounce
4	Moderately toxic	500 to 4,999	1 ounce to 1 pint (1 pound)
5	Slightly toxic	5,000 to 14,999	1 pint to 1 quart
6	Practically nontoxic	15,000 and above	More than 1 quart

Adapted from Heikes 1967; Hodge and Sterner 1949; Klaasen and Doull 1980; and Loomis 1978.

considered "dangerous" (or highly toxic). Similarly, substances with an LC_{50} between 1 and 10 ppm should be considered "harmful" (toxic) and those with an LC_{50} above 10 ppm should be considered "slightly toxic." The toxicity of chemical substances to fish varies widely with substance, fish species and age, and environmental variables. Table 2-3 illustrates the range of toxicities of various substances to young rainbow trout when tested in water at 50 to 55.4°F.

Table 2-3

Comparative Acute Toxicities of
Selected Substances to Rainbow Trout[a]

Substance	Principal Use	96-Hour LC_{50} (ppm)	95% Confidence Limits (ppm)
Acephate	Insecticide	1,100	775-1,561
Fire-trol 931	Fire Retardant	940	796-1,110
Ureabor	Herbicide	640	387-1,057
Glyphosate	Herbicide	130	108-156
Simazine	Herbicide	100	not given
Mirex	Insecticide	100	not given
Propham	Herbicide	38	34-43
Aminocarb	Insecticide	13.5	11.3-16.2
Diuron	Herbicide	4.9	4.1-5.9
Vernolate	Herbicide	4.3	3.9-4.7
Alachlor	Herbicide	2.4	1.8-3.1
Fenitrothion	Insecticide	2.4	2.0-2.9
Carbaryl	Insecticide	1.95	1.45-2.63
Coumaphos	Insecticide	0.89	0.65-1.15
Benomyl	Fungicide	0.17	0.12-0.23
DDD	Insecticide	0.07	0.057-0.087
Chlordane	Insecticide	0.042	0.037-0.048
Trifluralin	Herbicide	0.041	0.026-0.062
DDE	Insecticide	0.032	0.026-0.040
Lindane	Insecticide	0.027	0.020-0.036
Toxaphene	Insecticide	0.011	0.079-0.013
DDT	Insecticide	0.0087	0.0068-0.0114
Antimycin A	Picicide	0.000012	0.000007-0.000023

[a]Data from 0.8 to 1.5 gram fish tested at 50 to 55.4°F (10 to 13°C) using technical material containing from 93 to 100% active ingredient.

Source: Johnson and Finley 1980.

3.0 Fate and Transport of Pesticides

In natural environments, pesticides are transported through various environmental compartments, stored, and/or degraded or otherwise inactivated by a variety of biological, physical, and chemical mechanisms. The fate and transport of any pesticide depends upon the physical and chemical properties of both the pesticide and the environment.

The ability of a pesticide to be transported within the environment depends primarily upon its vapor pressure, its solubility in water, and its adsorption to soil particles. Pesticides with high vapor pressures are volatile and are very mobile in the gaseous state. Unless techniques such as soil incorporation are used during application, such pesticides are readily transported through air. Highly water soluble pesticides, that are not otherwise bound to soil particles, can be carried into waterbodies such as rivers and streams by overland runoff following precipitation or as a result of leaching by underground water. Pesticides that are strongly adsorbed onto soil particles are not readily transported, nor are they readily available for uptake by biological organisms, in many instances.

Some pesticides tend to accumulate in animals and/or plants, while others do not. Highly toxic chemicals that bioaccumulate present a greater degree of potential harm to animals that eat the affected plants and/or prey species than chemicals that do not bioaccumulate.

Finally, pesticides may be degraded in soil and water by biological, physical, and chemical processes. Pesticides are subject to biodegradation, metabolic detoxification and decomposition by organisms, or to photodegradation. Many are degraded, to varying degrees, by light.

Each of the individual Herbicide Background Statements addresses environmental fate and transport of the subject herbicide in terms of bioaccumulation and metabolism in plants and animals, behavior in soils, and behavior in aquatic environments. Available experimental data obtained from animal bioaccumulation and metabolism studies are summarized in the text and presented in more detail in Appendix F in each of the individual Herbicide Background Statements. Behavior of each herbicide in soil is discussed in terms of persistence, mobility, adsorption, chemical and microbial degradation, and volatilization. Behavior in water is similarly discussed in terms of persistence, mobility, and degradation.

3.1 Bioaccumulation and Metabolism

An understanding of the ultimate fate and transport of chemicals in the environment can only be reached with some knowledge of how

readily these chemicals accumulate in living organisms as well as how readily they are metabolized or eliminated from plants and animals. Such information is important for toxic substances that tend to bioaccumulate in food-chain organisms as well as for substances that are metabolized in plants or animals to toxic metabolites.

The degree to which a chemical substance is accumulated by biological systems depends upon a variety of factors, including solubility in organic solvents, solubility in water, rate of excretion by organisms, and rate of metabolism into toxic or nontoxic products. Solubility of the chemical substance in organic solvents influences its ability to pass through cell walls, mucus membranes, cuticle, and/or other surfaces of an organism and to remain in cellular or interstitial fluids. The solubility of substances in water influence the degree to which they are available for uptake by aquatic organisms. Animals accumulate toxic chemical substances by eating food containing a body burden of such substances. Accumulation of toxic substances in such organisms is influenced to a large extent by the amount of substance in the food organisms, as well as the degree to which such substances tend to be stored in tissues rather than be excreted or metabolized.

Weber (1977) compared bioaccumulation for a wide variety of pesticides, ranging from a factor in oysters of 70,000 for DDT to 0 for carbaryl (also in oysters). For comparative purposes, Weber proposed a classification of pesticides based on bioaccumulation, presented in Table 3-1.

Many organisms metabolize pesticides, either into other toxic substances, nontoxic materials that are stored, nontoxic materials that are used for normal metabolic purposes, or materials that are readily excreted by the organism. These metabolic processes are generally collectively referred to as biotransformations.

3.2 Soil

The behavior of a chemical substance in soil is determined by a number of properties relating to both the chemical and the soil environment. Properties of the chemical include water solubility, adsorption onto soil particles, chemical degradation, degradation by soil organisms, and volatility. Environmental properties include precipitation levels as well as soil type, organic matter content, temperature, and pH. Chemical substances that are highly soluble in water tend to be readily leached from soil, particularly if there are large amounts of precipitation and/or the chemical does not tend to adsorb readily to soil particles.

Table 3-1

Classification of Pesticides Based on Bioaccumulation Factor

Classification	Bioaccumulation Factor[a]	Characteristics
Nonaccumulative	10 or less	Pesticides readily decreases when organism is removed from exposure.
Slightly accumulative	60 to 700	Pesticide is only gradually lost when organism is removed from exposure, or pesticide is degraded by the organism.
Moderately accumulative	700 to 8,000	Pesticide is only gradually lost when organism is removed from exposure, or pesticide is slowly degraded by the organism.
Highly accumulative	8,000 or more	Pesticide is not lost when organism is removed from exposure, or pesticide is not significantly degraded by the organism.

[a]Bioaccumulation factor is the ratio of the concentration of chemical substance in tissues of an organism to the concentration in the environment. A bioaccumulation factor of 1 indicates the same concentration in both organism and environment.

Source : Adapted from Weber 1977

Adsorption of chemical substances to soil particles depends on a number of factors. Chemical substances tend to adsorb more readily to soils with higher organic matter content than to soils with low content of organic material. Adsorption rates decrease with increasing temperatures and increased soil moisture content. Soil pH has a strong influence on adsorption of chemical substances. The processes of adsorption of pesticides on soil have been reviewed in detail by Hamaker and Thompson (1972).

Chemical substances in soil are subject to degradation by chemical and biological means, as well as to movement by diffusion, dissolution, or vaporization. Chemical degradation may occur by photolysis if the substance is exposed to light at wavelengths causing its photodegradation. Many soil microorganisms are capable of metabolizing a wide variety of chemical substances and, in soils with environmental conditions fostering microbial growth, biological degradation is an important means by which substances are removed from the soil. Volatile substances are capable of moving through the soil by diffusion and of vaporizing into the atmosphere. Finally, chemical substances that are soluble in water are leached into deeper soil zones or carried by overland flow into adjacent waterbodies.

3.3 Water

The behavior of chemical substances in water is influenced by the same factors of water solubility, adsorption onto sediment, chemical and microbial degradation, and environmental conditions that influence their behavior in soils (Norris et al. 1983). Chemical substances, particularly agricultural chemicals such as pesticides, can enter aquatic systems in various ways, including direct application to surface waters, drift from spray operations close to surface waterbodies, mobilization of residues in ephemeral stream channels, overland flow, and leaching. The relative contribution of chemicals to waterbodies by any of these routes results from an interaction of the properties of the chemical and specific environmental factors. Generally, chemical substances that enter waterbodies are rapidly diluted, either by the large relative volumes of water in lakes and ponds, or by the addition of stream and overland flow in stream systems.

For the most part, chemical substances used in forest management applications rarely reach high concentrations in aquatic systems. The substances are rapidly diluted, are adsorbed onto sediments, or are metabolized or bioaccumulated by aquatic organisms.

4.0 Assessment of Hazards

Toxicity is an inherent property of herbicides, which are designed to kill living organisms, specifically vegetation. However, misuse of herbicides may also cause adverse effects on nontarget organisms, such as humans and other animals. Consequently, it is important to assess the potential hazard that could be incurred following exposure of these nontarget organisms to specific herbicides.

Proper evaluation of the hazards associated with each herbicide requires data on the toxicity of the compounds and on the level of exposure to the compounds. In practice, the toxicity of the herbicides is assessed through controlled laboratory tests, which are conducted to determine the quantity of herbicides required to produce specific toxic effects. Laboratory data can then be compared to human exposures estimated for USDA Forest Service uses to indicate if actual use practices for a particular herbicide are likely to result in adverse effects to man or the environment.

The toxicity of noncarcinogens is assessed in terms of the no observable effect level (NOEL), while the toxicity of carcinogens is assessed in terms of potency. NOEL's are not used to assess the toxicity of carcinogens since there is evidence that there is no finite dose, or threshold, of a carcinogen that is without potential harmful effect. In the absence of a scientific consensus concerning thresholds for carcinogens, regulatory considerations adopt the conservative assumption that such thresholds do not exist.

The quantities of herbicides required to produce toxic effects are measured either in terms of the level of exposure or in terms of dose. Theoretically, dose is the actual quantity absorbed by the test animal. In actual practice with oral studies, it is generally assumed that the amount of material administered to the animal is the amount absorbed. Thus, in oral studies, the administered quantity is usually expressed as the dose. Rarely, except in metabolism studies, is the actual amount of absorption quantified.

Although the assessment of hazards is simple in principle, the necessary data are not easy to obtain, especially with regard to exposures or absorbed quantities. Actual exposure in the field is difficult to assess since application procedures vary widely, environmental and climatological conditions differ between sites, and very few studies have been conducted to determine the actual levels of exposure resulting from on-site applications of herbicides. Similarly, few studies have been conducted to determine absorbed quantities. However, based on a few exposure studies conducted during application, absorption quantities can be estimated as a

percentage of the exposure level. In preparing the individual Herbicide Background Statements, a number of these estimates or assumptions have been made. The general and specific assumptions used to calculate exposures are described below in detail, and are used in each Herbicide Background Statement.

4.1 General Assumptions

It is USDA Forest Service policy to use only those pesticides registered by the Environmental Protection Agency. It is assumed throughout the Herbicide Background Statements that this policy is followed. Assessments of hazards associated with nonlabelled uses or application rates were not considered.

The organisms considered for hazard assessment in the Herbicide Background Statements include (at a minimum) humans, deer, rabbits, and fish. Deer represent a valuable environmental and recreational resource. Rabbits represent a worst case for exposure due to their relatively high ratio of surface area to body weight and their relatively small size, which results in a proportionately higher food intake compared to larger mammals. Fish were considered because herbicides can contaminate recreational waters. Hazard assessments for all organisms, with the exception of herbicide applicators (occupational), were conducted using estimated exposure data according to the methodology described in Sections 4.2.2.

Organisms may be exposed to herbicides by dermal means (skin contact), by inhalation (breathing), and by ingestion (eating or drinking). These three routes of exposure include virtually all exposure situations. Fish may be considered to be exposed by immersion (a combination of the routes).

The hazard assessments in the individual Herbicide Background Statements consider occupational doses and environmental exposures. Occupational doses, which refer to the dose incurred by workers during herbicide application, were used for the basis of worker hazard assessment because of the availability of experimental data on doses incurred by phenoxy herbicide applicators. Specific assumptions involved in calculating occupational doses are given in Section 4.2.1. Environmental exposures estimated for non-occupational hazard assessments are used to permit comparisons with toxicological data based on known exposure levels. Environmental exposures refer to those exposures incurred directly by persons entering a forest after application operations have ceased, as well as those incurred indirectly by persons who consume animals and vegetation indigenous to the sprayed forest. Exposures incurred by representative animals (deer, rabbits, and fish) are also considered as environmental exposures for the purposes of these analyses.

Environmental exposures to humans from ingestion of contaminated material were calculated using explicitly stated assumptions for each route of exposure. No attempt is made to convert these exposure values to doses (actual absorption), since the laboratory toxicity data used for comparison are based on exposure levels rather than dose. However, in the case of oral ingestion of animal and plant material by humans, it is necessary to derive animal and plant tissue concentrations of the herbicides so that human exposure levels from such ingestion can subsequently be calculated. Calculations were based on a 50 kg person.

Occupational doses and environmental exposures are assumed to be directly related to application rate for any given herbicide. Thus, if the application rate is doubled, the occupational dose and environmental exposures are also expected to double. Within each Herbicide Background Statement, maximum reasonable occupational doses and environmental exposures are calculated. These calculations are based upon actual Forest Service application rates multiplied by occupational dose and environmental exposure factors based on a 1 lb a.e. or a.i./acre application rate. These dose and exposure factors, and the detailed assumptions involved in their derivation, are presented in Section 4.2 below.

It should be noted that the calculations that follow in Section 4.2 of this General Introduction, as well as the calculations in Section 4 of each of the individual Herbicide Background Statements, were based on available information from various sources. The precision of measurement in the original data or estimates varied. However, to generate comparative data regarding occupational doses and environmental exposures, assumptions and mathematical operations were applied consistently for each chemical, but this resulted in indicated degrees of accuracy and precision unwarranted by the original data. For example, application data were measured to the nearest 0.1 lb a.i./acre. Estimates such as herbicide concentrations in mushrooms, on the other hand, were 1 percent of 5 ppm. This resulted in estimates at the 0.01 ppm level, which were further manipulated to estimate human exposure at the 0.0001 ppm level per pound applied per acre. Although human exposure was not actually measured to the nearest ten-thousandth ppm, the calculation is useful in indicating the approximate level of expected exposure. Similarly, calculated exposure levels of 0.005 mg/kg and 0.0002 mg/kg for two particular compounds may not be indicative of differences in actual measurement precision, but rather may result from the mathematical operations conducted. No attempts were made to round down such values, since it was important to discern the relative differences between all means of exposure.

4.2 Specific Assumptions

4.2.1 Occupational Doses

Occupational exposures or doses incurred by workers during application of most of the herbicides reviewed in the individual Herbicide Background Statements have not been reported in the literature. Lavy and coworkers (Lavy 1978, 1980; and Lavy et al. 1983) have attempted to quantify occupational exposures and absorbed doses of several herbicides (primarily 2,4-D and 2,4,5-T) based on urinary output, sampling of air, and deposition on patches. Results of these studies indicated that, although workers were exposed to herbicides, the levels of exposure were below any amounts that produce toxic effects in laboratory animal studies. The major route of exposure was dermal. Lavey (1980) estimated average 2,4-D doses ranging from 0.00049 mg/kg body weight for observers to 0.0198 mg/kg body weight for pilots and mixer/loaders during application at 2 lb a.e./acre with ordinary safety precautions. These doses were significantly reduced when special precautions, such as disposable clothing and stringent hygienic practices, were employed. For 2,4,5-T, Lavey (1978) found average absorbed doses of 0.002 mg/kg body weight in flagmen and 0.045 mg/kg body weight in mist-blower operators at an application rate of 2 lb a.e./acre and an average absorbed dose of 0.063 mg/kg body weight in backpack applicators at an application rate of 1.6 lb a.e./acre.

In the individual hazard assessments, occupational doses are considered for three worker categories: mixer/loaders; observers; and backpack sprayers. For mixer/loaders and backpack sprayers, maximum reasonable occupational doses were derived from urinary output studies with workers based on normal procedures and work clothes. For observers, estimates of maximum reasonable occupational doses were based on assumptions of exposure and dermal absorption to account for the possibility that an observer may accidentally be overflown by an aerial spray craft. In the absence of comparable data on occupational doses, by worker category, for all of the herbicides of concern, occupational doses derived for phenoxy herbicides (2,4-D and 2,4,5-T) were assumed to be reasonable surrogates for assessing hazards from application of other herbicides. Determination of occupational doses, based on an application rate of 1 lb a.e. or a.i./acre for each of the three worker categories was derived as follows:

Mixer/loader. Urine analysis with 2,4-D indicated an occupational dose of 0.0198 mg/kg in mixer/loaders in a study using ESTERON® 99 (4 lb a.e. 2,4-D/gallon concentrate) applied at 2 lb a.e./acre under normal operating procedures (Lavy 1980). Doses of 0.1 mg/kg body weight were reported in a

mixer/loader during 2,4,5-T application (Lavy et al. 1980 in U.S. Department of the Interior 1983 and Lavy 1978).

For the hazard assessments in the individual Herbicide Background Statements, a dose of 0.1 mg/kg body weight/day was assumed for mixer/loaders based on the maximum value reported by Lavy and his coworkers. Since the mixer/loaders handle concentrated formulations, this dose level was assumed to be independent of application rate, and was therefore constant for all the herbicides unless data, such as increased rates of dermal absorption, indicate otherwise.

Observer. Based on urine analysis during 2,4-D application, Lavy (1980) estimated average occupational doses of 0.00231 mg/kg and 0.00049 mg/kg for supervisors and observers, respectively, at an application rate of 2 lb a.e./acre. In a similar study with 2,4,5-T, he estimated an average dose for flagmen to be 0.0777 mg/kg with an application rate of 2 lb a.e./acre.

Since accidental direct exposure of observers is possible as part of a maximum reasonable exposure scenario, an occupational dose based on theoretical calculations was derived that is higher, and thus more conservative, that the values derived by Lavy (1978 and 1980). It was assumed that a 50 kg observer receives one direct exposure per day, that the area of exposed skin is 2 ft^2, and that the dermal absorption rate of the herbicide is 10 percent. (Feldmann and Maibach [1974] reported a dermal absorption rate of 5.8 percent for 2,4-D.) Thus, for every pound of active ingredient applied per acre, approximately 10 mg will be deposited on each square foot of surface area (454 g/lb x 1000 mg/g x 1 lb/acre x 1 acre/43,560 ft^2). This is a conservative estimate, since the exposed body surface is neither flat nor exposed horizontally to the falling herbicide deposit. Consequently, the theoretical-maximum reasonable dose for the observer is 0.04 mg/kg/day (10 mg/ft^2 x 0.1 x 2 ft^2/man x man/50 kg) for each pound of herbicide applied per acre.

Backpack Sprayer. Based on urine analysis during 2,4,5-T application in the fall in south central Arkansas, Lavy (1978) estimated an average absorbed dose of 0.063 mg/kg for backpack sprayers at an application rate of 1.6 lb a.e./acre. This dosage is equivalent to 0.04 mg/kg per 1 lb a.e. applied per acre.

In summary, the occupational doses assumed for the Herbicide Background Statements were as follows:

Mixers/loaders - 0.1 mg/kg/day, independent of application rate

Observers - 0.04 mg/kg/day for each pound a.i. applied/acre

Backpack sprayers - 0.04 mg/kg/day for each pound a.i. applied/acre

4.2.2 Environmental Exposures

Environmental exposures to herbicides in humans and other animals occur through all major routes of exposure. Such exposures result from spray drift, runoff, and accumulated concentrations of herbicides in water, vegetation, and meat. Exposure decreases with time after application due to degradation and/or loss by transport or dilution of herbicide. Environmental exposures to herbicides are discussed in the Herbicide Background Statements in terms of route of exposure. Calculations were made for the organisms of concern for each route of exposure, where appropriate, based on available data and/or reasonable assumptions regarding application practices and organism behavior.

Visitors to the forest, or persons downwind of the application site, may be exposed to herbicides by dermal contact, inhalation, or ingestion. However, dermal and inhalation exposures of non-applicators were considered to be low relative to those experienced by applicators. For example, a casual visitor to spray units would receive a dermal exposure much lower than that of workers involved in application of the herbicide. Consequently, occupational exposures to the herbicides were considered to be a maximum reasonable exposure, and direct environmental exposures to humans by dermal contact and inhalation were not considered further. However, since any human may eat vegetation, wildlife, or fish that may contain herbicide residues, direct environmental exposure from ingestion was considered. Consequently, all routes of exposure were considered for animals that may be eaten by humans.

Calculations of environmental exposures that would result from spray drift outside of the target area, such as drift into waterbodies or onto vegetation that may be eaten by humans, require consideration of spray drift. For herbicides applied aerially in a 5 mph wind, concentrations of spray drift 100 feet downwind are approximately 1 percent of those onsite (U.S. Department of the Interior 1983).

Dermal

Dermal exposure to herbicides for an animal in the spray zone is a function of the deposition rate on the animal, of the surface area

contacted by the deposited spray, and of the actual amount of dislodgeable residues that actually penetrate the pelage and thus contact the skin surface. Smaller animals represent a proportionately larger surface area per unit weight. Therefore, rabbits represent game mammals with a relatively high potential exposure on a per unit weight basis compared to deer.

Actual exposures are difficult to estimate. For example, forest characteristics and animal behavior may affect deposition rate or contact area. Specifically, herbicide deposits on the forest floor were estimated at approximately one-third of the overstory canopy deposit (Newton and Dost 1981), or 35 mg/m^2 at 1 lb/acre application, since aerially applied sprays are partially intercepted by overstory foliage. In addition, aircraft overflights normally frighten game into seeking cover. Rabbits will likely seek their burrows. It is not anticipated that deer will run into open-area spray clouds, but will move away from spray craft or into covered areas. Since such events cannot be assumed to prevent all exposure, conservative assumptions were made.

For dermal exposures, the following assumptions were made:

- deer were estimated to weigh 80 kg and have 2.3 m^2 surface area;
- rabbits were estimated to weigh 2.0 kg and have 0.23 m^2 surface area;
- the total body surface of the animal is equally exposed to spray, either from direct deposit or contact with treated foliage as the animal walks through it; and
- 10 percent of the deposited spray actually penetrates the pelage resulting in dermal exposure.

The estimated dermal exposure to deer is 0.101 mg/kg (35 mg/m^2 x 2.3 m^2/deer x deer/80 kg x 10 percent) for each pound of active ingredient applied per acre. The estimated dermal exposure to rabbits is 0.403 mg/kg (35 mg/m^2 x 0.23 m^2/rabbit x rabbit/2 kg x 10 percent) for each pound of active ingredient applied per acre.

Deer dermal exposure - 0.101 mg/kg Rabbit dermal exposure - 0.403 mg/kg	} for each pound a.i. applied per acre

Inhalation

Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time

measured in minutes. Exposure would result from inhalation of spray droplets of respirable size as well as from herbicide vapor for those substances with a high vapor pressure. Exposure by this route is expected to be of such short duration that it can be neglected.

Immersion

Exposure to fish was based on the assumptions that a 100-foot buffer zone would be maintained adjacent to streams and ponds during herbicide application and that the minimum average water depth would be 6 inches. Using the assumed buffer zone, the downwind concentration of herbicide applied in a 5 mph wind would be approximately 1 percent of the on-target concentration (U.S. Department of the Interior 1983). Thus, for each pound of active ingredient applied per acre, the concentration 100 feet from the target site would be 0.1 mg/ft^2. For a waterbody with a depth of 6 inches, the resulting concentration would be 0.2 mg/ft^3, or 7.067 mg/m^3, for every pound of active ingredient applied per acre. This is equivalent to a concentration of 0.007 ppm (7.067 mg/m^3 x m^3 water/10^9 mg water) for every pound of active ingredient applied per acre. To be conservative, this figure was rounded upward and exposure to fish was assumed to be 0.01 ppm per lb a.i. applied per acre. This estimate does not include the presence of residues that may reach waterbodies via runoff. However, the estimated concentration of 0.01 ppm for 1 lb a.i. applied per acre is higher than that of 99 percent of sampled streams following actual upstream phenoxy herbicide application (Neisess 1983).

Oral

Humans and other animals may be exposed to herbicides through oral ingestion of contaminated materials. Animals may ingest plant material and water and may also be exposed because of grooming or fur licking. Humans may ingest vegetation (e.g., berries and mushrooms), game, fish, and water. Since herbicides are usually rapidly degraded or, if not degraded, translocated to often inedible plant parts, exposure by ingestion of plants in humans and other animals will be only a short-lived phenomenon. Similarly, exposure by ingestion of game, fish, and water will also be short-lived, as will animal exposure due to licking of fur.

Oral exposure of animals to aerially applied pesticides due to ingestion of water was not considered in the background statements, since estimated water contamination, as calculated, would amount to 0.01 ppm per lb a.i. applied per acre for 6-inch deep watercourses. This concentration would be less for deeper waterbodies and is several orders of magnitude less than food concentrations. Animals may consume some amounts of herbicide if they lick dewfall from

foliage that has recently been sprayed; however, such exposure due to ingestion has not been adequately quantified in field studies. In addition, oral exposure due to ingestion of herbicide by licking of fur has not been quantified in field or laboratory studies.

Animal exposures due to ingestion of contaminated plant material were calculated separately for deer and rabbits due to differences in browsing patterns in these animals. It was assumed that deer browse at forest edges where foliage is maximally exposed to herbicides following aerial spraying, whereas rabbits were assumed to eat foliage that is at least partially protected from deposition by overstory vegetation. Throughout the background statements, the application rate that was greatest (i.e., either aerial or ground) was used to show worst-case situations. Absent specific data, it was assumed that exposure would last 2 days.

Deer exposure calculations assume the following:

- post-spray concentration of herbicide in browse would be, as a maximum reasonable case, 100 ppm per lb of herbicide applied per acre (Newton and Dost 1981) and

- a typical deer weighs 180 lbs (80 kg) and eats 2.5 lb (1.15 kg) field weight foliage per day (Moen 1973)

Thus, daily exposure for deer due to ingestion of herbicides would be 1.44 mg/kg (100 mg/kg x 1.15 mg/deer x deer/80 kg) for each pound of active ingredient applied per acre.

Rabbit exposure calculations assume the following:

- herbicide concentrations in food foliage would be 35 ppm per lb of herbicide applied per acre (Newton and Dost 1981) and

- a typical rabbit weighs 2.0 kg, eats 60 grams of dry food daily, and receives a dose of 0.03 mg/kg/day for each ppm in food (Lehman 1959 and Table 5-2 of this introduction)

Thus, rabbits would have a daily exposure due to ingestion of herbicides of 1.05 mg/kg (35 ppm x 0.03 mg/kg/day/ppm) for each pound of active ingredient applied per acre.

Deer Oral Exposure - 1.44 mg/kg	} for each pound a.i. applied per acre
Rabbit Oral Exposure - 1.05 mg/kg	

Oral exposures to humans occur through ingestion of fish, meat, vegetation, and water. The levels of exposure would be a function of

herbicide concentration in the food or water as well as the quantity of material consumed. Furthermore, not all of the ingested material would be assimilated once it is eaten by the human.

Estimates of oral exposure for human consumption of fish, which are exposed through immersion, have the following assumptions for a maximum reasonable case:

- streams and ponds containing fish will be protected from aerial spray by a 100-foot buffer zone and will be at least 6 inches deep;

- herbicide concentrations in water beyond the buffer zone are, at a maximum, 1 percent of on-target concentrations, or 0.01 ppm per pound of active ingredient applied per acre;

- a bioconcentration factor of one exists unless specific data to the contrary are available;

- herbicide residues are evenly distributed in fish tissue unless otherwise indicated by specific data;

- fish with the highest tissue concentrations are most likely to be in shallow streams and ponds and thus would be too small to be edible; and

- fish catch would be 1 kg per day for two days, with 50 percent of the fish biomass eaten over a two-day interval.

Consequently, concentrations in fish tissue were estimated to be 0.01 ppm, (0.01 ppm x 1), which is equivalent to 0.01 mg/kg, for each pound a.i. applied per acre. Human consumption of 0.5 kg of fish for two days would result in an oral human exposure of 0.0002 mg/kg (0.01 mg/kg x 0.5 kg/day/person x 2 days x person/50 kg).

Human exposure from eating fish - 0.0002 mg/kg for each pound a.i. applied per acre.

Oral exposure estimates for human consumption of both deer and rabbit meat make the following assumptions as a maximum reasonable case:

- deer and rabbit meat will come from game that may have been in the target spray zone during herbicide application and that may have eaten sprayed vegetation;

- residues of herbicide in the meat result from both oral and dermal exposure of the game, which in turn result from the level of exposure of the game to the herbicide, assimilation of the herbicide, and duration of exposure;

- assimilation by deer and rabbit is 10 percent of exposure for both dermal and oral routes unless otherwise indicated by specific data to the contrary;

- herbicide residues would be evenly distributed in meat tissue, unless otherwise indicated by specific data; and

- maximum human daily consumption of game totals 0.5 kg per day over a two-day interval.

Deer tissue concentrations were determined by combining the maximum estimated doses resulting from dermal and oral exposures. Since dermal exposures in deer were previously determined to be 0.101 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure is estimated to be 0.01 mg/kg (0.101 mg/kg x 0.1). Since daily oral exposures were previously determined to be 1.44 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure was estimated to be 0.288 mg/kg (1.44 mg/kg/day x 2 days x 0.1). Thus, total deer tissue concentration was the sum of the two doses (0.01 mg/kg + 0.288 mg/kg).*

Total deer tissue concentration* - 0.298 mg/kg for every pound a.i. applied per acre

Since it was assumed that a person would eat as much as 0.5 kg of deer meat per day for two days, the human exposure resulting from eating contaminated deer meat would be 0.006 mg/kg (0.298 mg/kg/day x 0.5 kg/day/person x person/50 kg x 2 days). It should be emphasized that not all of the ingested material would be assimilated once it is eaten by the human.

Human exposure from eating deer* - 0.006 mg/kg for each pound a.i. applied per acre

Rabbit tissue concentrations were also determined by combining the estimated doses resulting from dermal and oral exposures. Since dermal exposures were previously determined to be 0.403 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure is estimated to be 0.04 mg/kg (0.403 mg/kg x 0.1). Since daily oral exposures were determined to be 1.05 mg/kg for each pound a.i. applied per acre, the dose resulting from this exposure was estimated to be

*These calculations assume that aerial application and ground application rates are the same. When the rates differ, deer and rabbit dermal doses are based on aerial application rates and the oral doses based on the higher of the two application rates.

0.21 mg/kg (1.05 mg/kg/day x 2 days x 0.1). Thus total rabbit tissue concentration is the sum of the two doses (0.04 mg/kg + 0.21 mg/kg).*

Total rabbit tissue concentration* - 0.25 mg/kg for every pound a.i. applied per acre

Since it was assumed that a person would eat as much as 0.5 kg of rabbit meat per day for two days, the human exposure resulting from eating contaminated rabbit meat would be 0.0005 mg/kg (0.25 mg/kg/day x 0.5 kg/person x person/50 kg x 2 days). As with deer meat, it should be emphasized that not all of the ingested material would be assimilated once it is eaten by the human.

Human exposure from eating rabbit* - 0.005 mg/kg for each pound a.i. applied per acre

Oral exposure estimates for human consumption of contaminated water assume the following:

- water sources adjacent to aerial spray zones will be protected by a 100-foot buffer and will receive a maximum drift deposition that is 1 percent that of the on-target deposition;

- water sources will have a minimum average depth of 6 inches, resulting in maximum herbicide concentrations of 0.01 ppm (.01 mg/liter) at 1 lb/acre application;

- human daily consumption of water is 2 liters;

- contaminated water will be consumed for only one day; and

- runoff into such waterbodies from precipitation events soon after spray application may result in brief increases in concentrations of herbicide; however, due to the transitory nature of these events and the relatively small contribution of drinking water to human exposure, runoff is unlikely to be a significant contributing factor for exposure and thus need not be considered in this analysis.

Thus, the human exposure to herbicides contributed by drinking water would be 0.0004 mg/kg (0.01 mg/liter x 2 liter/person/day x 1 day x person/50 kg). As with game, it should be emphasized that not all of the ingested material would be assimilated by humans.

*See previous page for footnote.

Human exposure from drinking water - 0.0004 mg/kg for each pound a.i. applied per acre

Oral ingestion of contaminated vegetation is considered in terms of berries and mushrooms collected at least 100 feet off of the target zone, where concentrations of herbicide on the plant material would be a maximum of 1 percent of those directly deposited on plant material within the target zone. Such an assumption assumes adequate posting of spray buffers and ignores the intentional disregard of such postings by forest visitors. The U.S. Department of the Interior (1983) has assumed a maximum of 10 ppm (10 mg/kg) herbicide on berries and 5 ppm (mg/kg) on mushrooms following aerial spray based on actual maximum measured values of phenoxy herbicides of 7 ppm in berries and 4.5 ppm in mushrooms. Specific data on spray and measurement of residues in these studies are not available. However, since reliable data on herbicide concentrations on berries and mushrooms are not available, these values are assumed in this analysis. A maximum daily consumption of 0.5 kg (for one day) of either berries or mushrooms was assumed. It was further assumed that collection would take place at least 100 feet off of the target zone where concentrations would be 1 percent of the on-target measurements, or 0.1 ppm (0.1 mg/kg) for berries and 0.05 ppm (0.05 mg/kg) for mushrooms. Implied are the assumptions that spray buffers will be adequately posted and that such posting will be obeyed by forest visitors. Thus, the human exposure to herbicides contributed by contaminated vegetation would be 0.001 mg/kg (0.1 mg/kg x 0.5 kg/person x person/50 kg) for berries and 0.0005 mg/kg (0.05 mg/kg x 0.5 kg/person x person/50 kg) for mushrooms. As with game and water, it should be noted that not all of the ingested material would be assimilated. In addition, much of the initially deposited material would be dislodged during picking or rinsed during washing.

Human exposure from berries - 0.0010 mg/kg
Human exposure from mushrooms - 0.00052 mg/kg
} for each pound a.i. applied per acre

4.2.3 Summary

The exposure and dose information calculated for animals and humans is summarized below, and results from an application rate of one pound a.i. applied per acre. An increase in the application rate is assumed to produce a proportionately higher number. All data are for one day, except for human ingestion of meat, which are for two days.

Occupational Doses

Mixers/loaders - 0.1 mg/kg (independent of application rate)

Observers - 0.04 mg/kg
Backpack sprayers - 0.04 mg/kg
} (per lb a.i. applied/acre)

Environmental Exposures for Animals (per lb a.i. applied/acre)

Dermal	Rabbit	- 0.404 mg/kg
	Deer	- 0.101 mg/kg
Immersion	Fish	- 0.01 ppm (water)
Oral	Rabbit	- 1.05 mg/kg
	Deer	- 1.44 mg/kg

Environmental Doses for Animals

Immersion	Fish	- 0.1 ppm (tissue)
Oral and Dermal	Deer	- 0.298 mg/kg*
	Rabbit	- 0.25 mg/kg*

Environmental Exposures for Humans (due to Ingestion)

Eating fish	- 0.0002 mg/kg
Eating rabbit	- 0.005 mg/kg*
Eating deer	- 0.006 mg/kg*
Drinking water	- 0.0004 mg/kg
Eating berries	- 0.0010 mg/kg
Eating mushrooms	- 0.00052 mg/kg

*These calculations assume that aerial application and ground application rates are the same. When the rates differ, deer and rabbit dermal doses are based on aerial application rates and the oral doses based on the higher of the two application rates.

5.0 Conversion Factors

A number of useful conversion factors are listed in Table 5-1. Ordinarily, to obtain the desired unit, one simply multiplies the known unit by a conversion factor. However, this is not always the case. For example, in a feeding study to convert parts per million (ppm) of a toxicant in the diet to mg/kg/day, it is necessary to know the weight of food consumed daily and the body weight of the animal under test. Approximate relationships of ppm to mg/kg/day for a variety of experimental animals are shown in Table 5-2. When the actual values for body weight of the animal and the weight of food consumed daily are known, then for 1 ppm in food the mg/kg/day is obtained by the formula:

$$\frac{\text{Grams Food Consumed/Day} \times 0.001}{\text{Body Weight in kg}} = \text{mg/kg/day for 1 ppm}$$

Table 5-1

Useful Conversions

Multiply	By	To Obtain
Gallon (U.S.)	3.785	Liters
Gallon (U.S.)	0.833	Gallon (Imperial)
Gallon (Imperial)	1.201	Gallon (U.S.)
Gallon (Imperial)	4.80	Quarts
Gallon	128.00	Fluid ounces
Gallon/acre	9.354	Liters/hectare
Hectares	2.471	Acres
Kilograms	2.205	Pounds
Kilograms/hectare	0.892	Pounds/acre
Kilometer	0.62	Miles
Liters/hectare	0.107	Gallons/acre
Miles	1.61	Kilometers
Ounces (fluid)	29.573	Milliliters
Ounces	28.35	Grams
Pounds	453.59	Grams
Pounds/gallon	0.12	Kilograms/liter
Pounds/acre	1.12	Kilograms/hectare

Table 5-2

Approximate Relation of Parts Per Million in Diet to mg/kg/day

Animal	Weight in Kilograms	Dry Food Consumed Per Day in Grams	1 ppm in Food Equals in mg/kg/day[a]	1 mg/kg/day Equals in ppm of Diet
Mouse	0.02	3	0.150	7
Chick	0.40	50	0.125	8
Young rat	0.10	10	0.100	10
Older rat	0.40	20	0.050	20
Guinea pig	0.75	30	0.040	25
Rabbit	2.0	60	0.030	33
Dog	10.0	250	0.025	40
Cat	2	100	0.050	20
Monkey	5	250	0.050	20
Dog	10	750	0.075	13
Man	60	1,500	0.025	40
Pig or Sheep	60	2,400	0.040	25
Cattle, maintenance	500	7,500	0.015	67
Cattle, fattening	500	15,000	0.030	33
Horse	500	10,000	0.020	50

a $\frac{\text{Grams Food Consumed/Day} \times 0.001}{\text{Body Weight in Kg}}$ = mg/kg/day for 1 ppm

Adapted from Lehman 1959.

6.0 Glossary

A number of terms and definitions that will be used in individual background statements are listed in Table 6-1.

Table 6-1

Glossary

Acceptable Daily Intake (ADI)	The maximum dose of a substance that is anticipated to be without lifetime risk to humans when taken daily.
Acid equivalent (a.e.)	The amount of active ingredient expressed in terms of the parent acid.
Active ingredient (a.i.)	The agent primarily responsible for the intended herbicidal effects of a product.
Adjuvant	Substance added to a spray to act as a wetting or spreading agent, sticker, penetrant, or emulsifier in order to enhance the physical characteristics of the herbicidal materials.
Adsorb	Adherence of a substance to a surface.
Bioassay	Determination of the quantity of a substance present by the use of sensitive indicator organisms.
Carcinogen	Any cancer-producing substance.
Carrier	Material added to an active ingredient to facilitate its preparation, storage, shipment, or use.
Chronic toxicity	The poisoning effects of a series of small doses applied over a long period.
Concentration	The amount of active ingredient or herbicide equivalent in a quantity of diluent, expressed as lb/gal, ml/liter, etc.

Table 6-1 (Continued)

Glossary

Congenital	Existing at birth but acquired in the uterus rather than inherited.
Cytological aberration	A deviation from normal cell structure or function.
Dose	A given quantity of test material that is taken into the body; quantity of material to be administered.
Dust	A dry, finely ground carrier material containing a pesticide active ingredient; used without further dilution.
Emulsifiable concentrate	A liquid formulation obtained by dissolving the technical active ingredient in a liquid solvent, and adding one or more emulsifiers, so that the formulated pesticide can be further diluted with water or oil for spray application.
Exposure	Application of test material to the external surfaces of a test organisms; takes into consideration route, duration, and frequency.
Formulation	(1) A pesticide preparation supplied by a manufacturer for practical use. (2) A manufacturing process by which technical active ingredients are prepared for practical use by mixing with liquid or dry diluents, grinding, or by the addition of emulsifiers, stabilizers, and other adjuvants.

Table 6-1 (Continued)

Glossary

Granular	A dry formulation of pesticide active ingredients mixed with or coated onto inert carrier material and other components in discrete particles generally less than 10 cubic millimeters in size. The carrier materials may be clays, sand, carbon, or ground corn cobs.
Hazard	The potential that the use of a pesticide would result in an adverse effect on man or the environment in a given situation.
Herbicide	A chemical used to control, suppress, or kill plants, or to severely interrupt their normal growth processes.
Intubation	Oral administration of a substance by means of a tube.
LC	Lethal concentration.
LC_{50}	The median lethal concentration; the concentration of toxicant necessary to kill 50 percent of the organisms being tested. It is usually expressed in parts per million (ppm).
LC_{100}	An LC which kills all of the test organisms.
LD_{50}	The median lethal dose; the size of a single dose of a chemical necessary to kill 50 percent of the organisms in a specific test situation. It is usually expressed in the weight of the chemical per unit of body weight (mg/kg). It may be fed (oral LD_{50}), applied to the skin (dermal LD_{50}), or administered in the form of vapors (inhalation LD_{50}).

Table 6-1 (Continued)

Glossary

LEL	Lowest effect level. In a series of dose levels tested, it is the lowest level at which an effect is observed in the species tested.
MLD	Minimum lethal dose; the smallest of several doses which kills one of a group of test animals.
Mutagenic	Capable of inducing a mutation. An agent that tends to increase the occurrence or extent of mutation.
Mutation	A new type of organism produced as a result of a heritable change.
NOEL	In a series of dose levels tested, it is the highest level at which no effect is observed, i.e., safe, in the species tested. It is the No Observable Effect Level
Nontarget vegetation	Vegetation which is not expected or not planned to be affected by the treatment.
Oncogenic (tumorigenic)	Capable of producing or inducing tumors in animals. The tumors may be either malignant (cancerous) or benign (non-cancerous).
Organogenesis	The time period during embryonic development during which all major organs and organ systems are formed. During this period, the embryo is most susceptible to factors interfering with development.

Table 6-1 (Continued)

Glossary

Pelleted formulation	A dry formulation of herbicide and other components in discrete particles, usually larger than 10 cubic millimeters.
Persistent herbicide	A herbicide which, when applied at the recommended rate, will harm susceptible crops planted in normal rotation after harvesting the treated crop, or which interferes with regrowth of native vegetation in noncrop sites for an extended period of time.
Pesticide	Any substance or mixture of substances intended for controlling insects, rodents, fungi, weeds, and other forms of plants or animal life that are considered to be pests.
Phloem	The living tissue in plants which functions primarily to transport metabolic compounds from the site of synthesis to the site of use.
Photodegradation	A process of breaking down a substance through reaction to light.
Phytotoxic	Injurious or lethal to plants.
Rate	The amount of active ingredient or acid equivalent applied per unit area or other treatment unit.
Residue	That quantity of herbicide, its degradation products, and/or its metabolites remaining on or in the soil, plant parts, animal tissues, whole organisms, and surfaces.

Table 6-1 (Continued)

Glossary

Residual herbicide	A herbicide that persists in the soil and injures or kills germinating weed seedings, over a relatively short period of time.
Risk	The probability that a substance will produce harm under specified conditions.
Safety	The reciprocal of risk, i.e., the probability that harm will not occur under specified conditions.
Selective pesticide	A chemical that is more toxic to some species than to others.
Soluble powder	Similar to a wettable powder, except that the technical active ingredient as well as the other formulation components will dissolve in water or other liquid for which the powder is formulated.
Spot treatment	A herbicide applied over a small continuous restricted area of a whole unit; i.e., treatment of spots or patches of brush within a larger field.
STEL	Short-term exposure limit. The maximal allowable concentration, or ceiling, not to be exceeded at any time during a 15-minute exposure period.
Surfactant	A material which favors or improves the emulsifying, dispersing, spreading, wetting, or other surface modifying properties of liquids.
Susceptibility	Capacity to be adversely affected by pesticide treatment.

Table 6-1 (Continued)

Glossary

Technical active ingredient	The active chemical along with unavoidable impurities remaining from the manufacturing process.
Teratogen	Any substance capable of producing structural abnormalities of prenatal origin, present at birth or manifested shortly thereafter (the ability to produce birth defects).
Threshold	A dose or exposure below which there is no apparent or measurable adverse effect.
TL_m (TL_{50})	Median tolerance limit; the concentration of chemical in water necessary to kill 50 percent of the test aquatic organisms during a specific exposure period. The TL_m is usually expressed as parts per million parts of water for 24, 48, 72, or 96 hours of exposure.
TLV-TWA	Threshold limit value-time weighted average. The time-weighted average concentration for a normal 8-hour workday and a 40-hour work week, to which nearly all workers may be repeatedly exposed without adverse effect.
Tolerance	(1) Capacity to withstand pesticide treatment without adverse effects on normal growth and function.

Table 6-1 (Continued)

Glossary

Tolerance (Concluded)	(2) The maximum residue concentration legally allowed for a specific pesticide, its metabolites, or breakdown products, in or on a particular raw agricultural product, processed food, or feed item. Expressed as parts per million (ppm).
Toxicity	(1) The capacity or property of a substance to cause any adverse effects. It is based on scientifically verifiable data from animal or human exposure tests. (2) That specific quantity of a substance which may be expected, under specific conditions, to do damage to a specific living organism.
Translocation	Movement of a pesticide or other substance within a plant via the phloem or xylem.
TWA	The time-weighted average concentration is the average exposure concentration based on the duration of exposure to airborne concentration as it varies during an 8-hour workday.
Weed control	The process of limiting weed infestations or killing weeds for aesthetic, economic, public health, or other reasons.
Weed eradication	The elimination of all live parts of plants and viable seeds of a weed from a site.
Weed suppression	The process of retarding weed growth.

Table 6-1 (Concluded)

Glossary

Wetting agent	A substance which serves to reduce interfacial tensions and causes spray solutions or suspensions to make better contact with treated surfaces.
Wettable powder	A finely ground, dry powder formulation that can be readily suspended in water for application in spray equipment.
Xylem	The non-living tissue which functions primarily to conduct water and mineral nutrients from the roots of plants.

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Herbicide Background Statements: Amitrole

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Amitrole Summary

Major Trade Names: Amizol®, Weedazol®, Amitrol-T®, Azalon®, Cytrol Amitrol-T®

Chemical Name: 3-Amino-1,2,4-triazole

Major Forestry Applications: Applied as a nonselective herbicide for conifer release, general weed control, range improvement, and rights-of-way maintenance.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Amitrole is a herbicide of very low acute toxicity and is generally nontoxic to a variety of nontarget organisms. Formulation with ammonium thiocyanate to increase its systemic action also increases its toxicity for several species. Studies with a variety of freshwater, marine, and terrestrial invertebrates demonstrated low acute toxicity (LC_{50} greater than 10 ppm) of amitrole. The acute oral toxicity for fish ranges from an LC_{50} of 10 ppm for amitrole to 10,000 ppm for Cytrol Amitrol-T®. The LD_{50} is greater than 2,000 mg/kg body weight for various species of birds.

Amitrole shows only very low toxicity in mammals following ingestion of single doses. The LD_{50}'s range from 25,000 mg/kg for rats to 14,700 mg/kg for mice. For Amitrol-T®, the acute LD_{50} in rats is 5,000 mg/kg. Mice, cats, or dogs were unaffected when amitrole was injected intravenously at dose levels of 1,600, 1,750, and 1,200 mg/kg, respectively.

Amitrole poses no hazard from dermal exposure. A single dose of 1,500 mg/kg applied to the skin of rats resulted in no signs of toxicity. The dermal LD_{50} for Amizol® was 10,000 mg/kg in rabbits. Eye irritation studies on experimental animals have not been reported. Subchronic toxicity studies show that amitrole affects primarily the thyroid gland resulting in adenomatous changes, hyperplasia, or goiter formation in rodents.

Amitrole is considered to be nonmutagenic, or a weak mutagen at best, since negative results were obtained in most of the mutagenicity assay systems. However, amitrole appeared to damage DNA as evidenced by the induction of unscheduled DNA synthesis in mammalian cells *in vitro*. Amitrole is not teratogenic in rats. In a multigeneration

reproduction study, pups born to parents fed high doses (500 and 1,000 ppm) of amitrole were runted and had atrophic thymuses and spleens. There was no runting at lower dietary levels, although hyperplasia of the thyroid was observed in all animals fed 100 ppm amitrole or greater.

Amitrole has been shown to be carcinogenic in animals, with induced tumors generally restricted to the thyroid gland in rats and mice. Benign and malignant liver tumors have been observed in rats and mice but no tumors were detected in dogs. An epidemiologic study of railroad workers in Sweden suggests that amitrole may be carcinogenic for man. However, the causal relationship between exposure to amitrole and tumor induction was unclear since workers were exposed to amitrole alone or with a combination of pesticides.

Environmental Fate

Amitrole is taken up by plants through both leaves and roots and is readily metabolized to a variety of compounds. In animals, amitrole is rapidly absorbed and eliminated, primarily in the urine, essentially as the parent compound but with small amounts of metabolites. It does not bioaccumulate in animals.

Amitrole has a very short persistence in soil (1 month or less) and is adsorbed to soil colloids. Although amitrole is biodegraded by organisms, it is primarily lost from soil by oxidation. In water, it tends to adsorb to sediments and is degraded by algae.

Exposure and Hazard

Estimated occupational and environmental exposures to amitrole from normal use are below those levels that would be expected to have acute or chronic toxic effects or reproductive effects. Although there is evidence that amitrole is carcinogenic in animals, there are insufficient epidemiological data concerning its carcinogenicity in humans. For forestry applications, the extremely small exposures and their short durations are considerably less than those that have caused toxicity and carcinogenicity in laboratory animals.

1.0 General Information

Amitrole (also known as aminotriazole and by the major trade names* Amitrol-T®, Cytrol Amitrole-T®, Weedazol®, Amino Triazole Weedkiller 90®, Herbizole®, Amizol®, Fenamine®, Fenavar®, and Kleer-Lot®) is a nonselective systemic herbicide originally patented in 1954 as a herbicide and plant growth regulator (Carter 1975 and Meister Publishing Company 1981). Studies reviewed by Carter (1975) indicate that amitrole affects a variety of biochemical processes in plants, including inhibition of amino acid and protein metabolism, interference with nucleotide and nucleic acid metabolism, and disruption of plastid development. In addition, amitrole is readily absorbed by both leaves and roots and is rapidly translocated up and down plant stems by xylem and phloem. Amitrole accumulates at the sites of new growth where its herbicidal effectiveness is increased (Carter 1975).

1.1 Normal Use Patterns

Amitrole was originally used for weed control around crops such as alfalfa, apples, clover, grains, grapes, legumes, pears, peas, and soybeans, as well as for weed control along fences, ditchbanks and highways. Amitrol-T®, a formulation of equal amounts of amitrole and ammonium thiocyanate, has been used for noncropland weed management and to control aquatic weeds such as water hyacinth (International Agency for Research on Cancer 1974). In 1959, however, amitrole was reported to induce thyroid tumors in rats and amitrole residues were found in marketed cranberries. Consequently, certain lots of cranberries and cranberry products from the 1958 and 1959 crops were withdrawn from the market and, in 1971, all registered uses of amitrole on food crops were canceled (House et al. 1967 in International Agency for Research on Cancer 1974 and Carter 1975).

Amitrole is used in forestry applications for conifer release, general weed control, poisonous plant control, range improvement, and rights-of-way maintenance. Data on the use of amitrole by the USDA Forest Service are presented in Table 1-1. Application rates for these uses range from an average of 1.7 lbs a.i. (active ingredient) per acre (1.9 kg/ha) for broadcast applications in general weed control to an average of 3.8 lb a.i./acre (4.6 kg/ha) for rights-of-way maintenance (Gross 1983). According to product literature summarized by the Weed Science Society of America (1979), amitrole is usually applied at rates of 2 to 10 lb a.i. per acre (2.2 to 11.2 kg/ha).

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service Amitrole Use

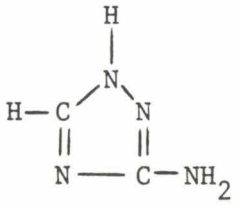
Purpose	Active Ingredient Per Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
1981					
Aerial					
Conifer release	1.8	1.8	482	Liquid	Helicopter
Ground					
Conifer release	0.4 - 3.0	2.3	119	Liquid	Individual plant
General weed control	1.7	1.7	60	Liquid	Broadcast
Poisonous plant control	1.1 - 2.2	1.9	73	Liquid	Individual plant
Range improvement	2.0	2.0	10	Liquid	Broadcast
Right-of-way	0.9 - 5.5	3.8	243	Liquid	Individual plant and Broadcast
1982					
Ground (14 projects)	0.5 - 4.7	2.9	505	Liquid	Ground foliar
Noxious Weeds, Range, Recreation, Wildlife Timber					

Source: Gross 1983.

Amitrole is marketed in the United States under a variety of trade names (Table 1-2). It is also formulated with various other herbicides. In general practice, Amitrol-T® formulations are diluted such that, for one acre of application, 1 to 2 gallons of formulation (1 gallon of formulation contains 2 lbs each of amitrole and ammonium thiocyanate) in 20 to 100 gallons of water are used for ground applications and 0.5 to 10 gallons of formulation in 5 to 15 gallons of water are used for aerial applications (Amchem product literature cited in U.S. Department of Agriculture 1981).

1.2 Physical and Chemical Properties

The active ingredient of the various amitrole formulations is 3-amino-1,2,4-triazole (also known as 3-amino-s-triazole) with the following structural formula (Weed Science Society of America 1983):



Amitrole has a molecular formula of $C_2H_4N_4$ and a molecular weight of 84.1. Pure amitrole consists of colorless crystals (when crystallized from water, ethanol, or ethyl acetate) with a melting point of 318°F (159°C). It is soluble in water (28 grams per 100 ml or 28 percent at 77°F [25°C]) and in ethanol (26 grams per 100 ml or 26 percent at 167°F [75°C]); only slightly soluble in acetonitrile, chloroform, ethyl acetate, and methylene chloride; and insoluble in acetone, ether, and hydrocarbons (International Agency for Research on Cancer 1974). Although the triazole ring structure around which the amitrole molecule is centered is stable to a wide variety of chemicals, it is cleaved by (OH•) or other free radicals. In the presence of riboflavin (a common vitamin [B_2] of plants and animals) and light, amitrole is oxidized, resulting in production of carbon dioxide, urea, cyanamide, and possibly nitrogen (Plimmer et al. 1967 in Carter 1975). This oxidation may be responsible for the rapid disappearance of amitrole from soils (Carter 1975). Amitrole will not decompose when heated to 212°F (100°C) for 2 hours. Under reduced pressure, amitrole crystals will sublime (evaporate) without decomposing. Aqueous solutions (solutions in water) of amitrole are neutral, but in the presence of acids, amitrole will act as a weak base to form salts.

In the presence of metal ions such as iron or copper, amitrole forms a ring structure incorporating the metallic ion (chelation) (Carter 1975). Consequently, amitrole is mildly corrosive to iron, aluminum, copper, and copper alloys. Equipment should be thoroughly flushed after use with amitrole formulations to avoid corrosion (Weed Science Society of America 1983).

Table 1-2

Amitrole Formulations[a]

Formulation	Active Ingredients	Formulator
Amitrol-T®	3-Amino-1,2,4-triazole 21.1%	Amchem
Cytrol Amitrole-T® Liquid Weedkiller	3-Amino-1,2,4-triazole 21.1%/w Ammonimum thiocyanate 19.1%/w Hydrochloric acid 9.8%/w	American Cyanamid
Weedazol®	3-Amino-1,2,4-triazole 50.0%	Amchem
Amino Triazole Weedkiller 90®	3-Amino-1,2,4-triazole 90.0%/w	American Cyanamid

[a]Formulations with other herbicides are omitted.

Source: Gosselin et al. 1976.

2.0 Toxicity of Amitrole

A summary of toxicological properties of amitrole (Table 2-1) shows that this herbicide is generally nontoxic to a variety of organisms. Studies with rodents indicate that amitrole is nonteratogenic and has little or no effect on reproduction. Amitrole has been shown to be carcinogenic in animals, and evidence shows it may be carcinogenic in man. It is nonmutagenic.

In the case of formulations in which ammonium thiocyanate have been incorporated to increase its systemic action (such as in Amitrol-T® and Cytrol Amitrole-T®), an increased toxicity has been observed in several species. In rats, an acute oral LD_{50} of ammonium thiocyanate is about 750 mg/kg body weight, while for amitrole, an acute oral LD_{50} in rats of as high as 25,000 mg/kg has been reported. For this reason, in the event of acute ingestion of amitrole formulations containing ammonium thiocyanate, ammonium thiocyanate poisoning rather than amitrole poisoning should be of concern (Weed Science Society of America 1983).

2.1 Invertebrates and Microorganisms

A variety of invertebrates (almost exclusively arthropods) and microorganisms from freshwater, marine, and terrestrial ecosystems have been studied for toxic effects of amitrole and amitrole formulated with ammonium thiocyanate. Major findings of these studies are presented in Appendix A and indicate a range of susceptibility to amitrole in the organisms tested demonstrating low toxicity (LC_{50} greater than 10 ppm).

For the most part, toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of amitrole in a natural environment. For example, in the laboratory, aquatic organisms were kept for as long as 4 days in water containing dissolved amitrole at various concentrations, and microorganisms were grown in culture media containing amitrole. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to amitrole or to other man-made chemicals is usually a transient phenomenon depending on streamflow, chemical dissipation, or decomposition. Additionally, in natural systems, interactions with other man-made and naturally occurring chemicals, as well as changes in environmental factors such as temperature and moisture, all potentially affect herbicide assimilation and metabolism by invertebrates and microorganisms and thus alter their tolerance levels.

Table 2-1

Summary of Toxicological Properties of Amitrole[a]

1. Acute Oral Toxicity

Organism (Formulation)	Adverse Effects
Rat (Amitrole)	LD_{50} = 1,100 to 25,000 mg/kg
Mouse (Amitrole)	LD_{50} = 14,700 mg/kg
Human (Amitrole)	No signs of intoxication after ingesting 20 mg/kg
Rat (Amitrol-T®)	LD_{50} = 5,000 mg/kg
Fish (various)	LC_{50} = 10 ppm (Amitrole) to 10,000 ppm (Cytrol Amitrol-T®)
Birds (Amitrole)	LD_{50} = 2,000 mg/kg

2. Acute Intravenous Toxicity

Organism (Formulation)	Adverse Effects
Mouse (Amitrole)	No effect at 1,600 mg/kg
Cat (Amitrole)	No effect at 1,750 mg/kg
Dog (Amitrole)	No effect at 1,200 mg/kg

3. Acute Dermal Toxicity

Organism (Formulation)	Adverse Effects
Rat (Amitrole)	No signs of toxicity at 2,500 mg/kg
Rabbit (Amizol®)	LD_{50} was 10,000 mg/kg

4. Eye Irritation

Organism (Formulation)	Adverse Effects
Human (Amitrole)	Eye injury reported after accidental exposure
Human (Amizol®)	Eye injury reported after accidental exposure

5. Subchronic Oral Toxicity

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rat (Amitrole)	2 weeks in diet	15 to 122 ppm	Thyroid enlargement.
Rat (Amitrole)	3 weeks	1,000 mg/kg I.P. (Intra-peritoneal)	Thyroid enlargement.

Table 2-1 (Continued)

5. Subchronic Oral Toxicity (Concluded)

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rat (Amitrole)	15 weeks	50 ppm in drinking water	Thyroid enlargement.
Mouse (Amitrole)	12 weeks	1% in diet	Thyroid hyperplasia, inhibition of whole body catalase enzyme activity.
Rabbit (Amizol®; 98% active ingredient)	25 weeks	0.2% in drinking water	Cataract formation; inhibition of lens catalase enzyme activity.

6. Teratology, Reproduction and Fertility

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Amitrole)	Multigeneration study. Animals fed 25 and 100 ppm	Runting of progeny, no teratogenic effects, hyperplasia of thyroid.
Rat (Amitrole)	20 or 100 mg/kg from days 7 through 15 of gestation	No reproductive or teratogenic effects.
Mouse (Aminotriazole)	500 to 5,000 ppm in drinking water from days 6 through 18 of gestation	No teratogenic effects.

7. Carcinogenicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Amitrole)	10 to 100 ppm in diet for 2 years	Thyroid adenomas and adenocarcinomas.
Rat (Amitrole)	20 to 25 mg/day in drinking water; 250 or 50 mg/day in diet for lifetime	Thyroid tumors; hepatocellular and hepatocholangiocellular carcinomas.

Table 2-1 (Continued)

7. Carcinogenicity (Continued)

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Amitrole)	Subcutaneous administration of 125 mg/rat twice weekly for 11 months.	Thyroid and liver tumors.
Rat (Amitrole)	Implantation of pellets under skin; 500 mg monthly for 5 months followed by 125 mg twice weekly for 6 months	Sarcomas at sites of treatment.
Mouse (Amitrole)	1,000 mg/kg by stomach tubes for 3 weeks; 2,192 ppm (330 mg/kg) in diet for 53 to 60 weeks	Carcinoma of thyroid; hepatomas.
Mouse (Amitrole)	0.1 or 10 mg applied to skin weekly for life	No skin tumors observed.
Dog (Amitrole)	10 to 500 ppm (0.75 to 37.5 mg/kg) in diet for 2 years	No tumors observed.
Man (Amitrole)	Railroad workers exposed amitrole alone or in combination with phenoxy herbicides for 46 or more days.	Increase in overall tumor morbidity and and mortality. Causal relationship unclear.
Virus-infected Rat embryo cells (Amitrole)	Cell cultures treated with 10 to 1,200 ppm	In vitro transformation.
Hamster embryo cells (Amitrole)	Cultures treated with 0.1 to 100 ppm	In vitro transformation.
Hamster kidney cells (Amitrole)	Cultures treated with 0.025 to 250 ppm	In vitro transformation.

Table 2-1 (Concluded)

7. Carcinogenicity (Concluded)

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Hamster kidney cells (Amitrole)	Cultures treated with 0.2 to 2,000 ppm	No *in vitro* transformation.

8. Mutagenicity

Microorganism	Adverse Effects
Salmonella typhimurium Strains TA98, TA100, TA1535, TA1537, TA1538	Nonmutagenic
S. typhimurium Strains TA1530, and TA1538	Equivocal results in host-mediated mutagenicity test
Aspergillus nidulans	Nonmutagenic
Escherichia coli	Nonmutagenic
E. coli bacteriophage	Nonmutagenic
Streptomyces coelicolor	Induction of resistance to streptomycin
Saccharomyces cervisiae	Inhibition of DNA repair
S. cervisiae, Strains JD1 and D6	Mutagenic
HeLa cells	Induction of unscheduled DNA synthesis
Fruit Fly (Insect)	**Adverse Effects**
Drosophila melanogaster	Nonmutagenic

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

Among the invertebrates tested (Appendix A), honeybees, representing insects of commercial value, exhibited a mortality of less than 5 percent when they were dusted with amitrole at rates equivalent to 12.09 μg/bee (Atkins et al. 1975 in Ghassemi et al. 1981). When bees were fed sugar water containing 250 ppm amitrole for six weeks, there were no changes in colony weight compared to controls, and no abnormalities attributable to amitrole were observed (King 1964).

Larval fruit flies raised in a medium containing amitrole exhibited a prolonged development time at concentrations as low as 10 ppm amitrole. The LD_{50} of amitrole to fruit flies, however, was 40 ppm in the culture medium (Laamanen et al. 1976). Amitrole was toxic to German cockroach nymphs (100 percent mortality) at levels of 1,000 ppm in food and it reduced the fecundity of adults (Riviere 1976).

Various studies with both marine and freshwater crustaceans indicated a variation in toxicity to amitrole formulations ranging from a 96-hour LD_{50} of 0.43 ppm (a.i.) for Amitrol-T® to larval copepod crustaceans (Bunting and Robertson 1975) to a 48-hour TL_{50} of >100 ppm for Amitrol-T® to such crustaceans as scuds, sowbugs, glass shrimp, and crayfish (Sanders 1970). Most studies with crustacea, however, resulted in median toxic levels greater than 10 ppm amitrole or Amitrol-T®, indicating that amitrole and its formulations as tested are only slightly toxic to crustacea.

In one study of field application of amitrole at a rate of 5 lb/acre (5.6 kg/ha), a 49 percent reduction in the number of soil nematodes was observed (Courtney et al. 1962 in Pimentel 1971). Whether this reduction in nematode numbers was due to toxic effects of amitrole, or to changes in the soil micro-habitat due to amitrole effects on plants, was not indicated.

The available information on the effect of amitrole and its formulations on microorganisms is somewhat ambiguous. Studies indicate that in conditions of minimal culture media, bacterial growth was inhibited or repressed at lower concentrations of amitrole than when a complete medium was used (Bamford et al. 1976) or when adenine (a precursor of DNA and RNA) and histidine (an amino acid) were added to the medium (Franco and Municio 1975). Cooper and co-workers (1978) cultured bacteria, actinomycetes, fungi, and yeast for 7 days in cultures containing 50 ppm of Weedazol-T® formulation. Only 2 percent of the actinomycetes cultures showed reduced or no growth with Weedazol-T®, while 27 percent of the fungal cultures had reduced or no growth. For bacteria and yeasts, 9 percent and 7 percent of the cultures were similarly affected in this study.

2.2 Fish and Amphibians

A wide variety of studies on amitrole, its formulations (Amitrol-T®, Weedazol TL Plus®, Cytrol Amitrole-T®), and ammonium thiocyanate, an ingredient in many amitrole formulations, have indicated LC_{50}'s greater than 10 ppm when tested with fish and amphibians (Appendix B). Clarke et al. (1970) suggest that pesticides with median lethal doses of 10 ppm or more for fish be considered "slightly toxic." Actual toxicities range from an amitrole $LC_{50} > 10$ ppm in mosquito fish (96-hour static bioassay; Johnson and Finley 1980) to a Cytrol Amitrole-T® LC_{50} of 10,000 ppm in bluegills (96-hour study; Cope 1963 in Pimentel 1971).

It has already been pointed out that ammonium thiocyanate has an acute oral toxicity to rats that is considerably greater than that of amitrole alone (Weed Science Society of America 1983). A Russian study reported by Springer (1957) in Pimentel (1971) indicated that 200 ppm of ammonium thiocyanate was lethal to "fish."

In a study of the toxic effects of Weedazol-TL Plus® to frog tadpoles, the 24-hour TL_{50} was 5,200 ppm and the 96-hour TL_{50} was 3,000 ppm (Johnson 1976).

At the high doses of amitrole that fish and amphibians have been subjected to in the tests described in Appendix B, a number of sublethal effects have been observed. Rainbow trout, fed 1,200 and 4,800 ppm amitrole in their diet, were reported to have a high incidence (up to 56 percent) of hepatomas (liver tumors) when autopsied after 9 months of feeding (Halver 1967 in International Agency for Research on Cancer 1974). Yearling coho salmon exhibited a dose-dependent mortality 24 to 48 hours after being transfered to sea water from freshwater containing Amitrol-T®, although there appeared to be no effect of the herbicide treatment on the osmoregulatory ability of the fish gills (Lorz et al. 1979). Frog tadpoles subjected to a dose of 1,000 ppm Weedazol TL Plus® (a subacute dose) for 96 hours had a reduced temperature tolerance compared to controls (Johnson 1976).

2.3 Birds

Dietary studies with amitrole indicate a low toxicity to birds (Appendix C). Japanese quail, ring-necked pheasants, and mallard ducks ingested as much as 5,000 ppm amitrole in their diet for 5 days without mortality (Hill et al. 1975). The acute oral LD_{50} for young male mallards (3 to 4 months old) was determined by Tucker and Crabtree (1970) to be in excess of 2,000 mg/kg body weight. In this latter study, the birds were observed to exhibit loss of coordination,

a slight wing drop, and weakness for the first 3 days after the acute dose. Mallards fed amitrole in doses 25 percent less than those that would normally lead to mortality suffered depressed reproduction (U.S. Department of the Interior 1962 in Pimentel 1971). When white leghorn chicks, 3 to 38 days old, were injected intraperitoneally with amitrole at doses as high as 1,000 mg/kg body weight, they displayed a reduced rate of weight gain and changes in the micro-anatomy of the thyroid gland (Wishe et al. 1979). Amitrole injected into embryonated chicken eggs at 100 ppm resulted in toxic effects (Dunachie and Fletcher 1970). Landauer et al. (1971) found dose-dependent abnormalities (primarily malformed beaks) in chicken embryos when the 96-hour embryonated eggs were injected with 10 to 40 mg amitrole per egg.

2.4 Mammals

The potential health hazards from exposure to amitrole have been evaluated primarily with laboratory animals such as mice and rats. No studies have been conducted on wildlife animals in the field.

Results of the various toxicity tests are described below and summarized in Table 2-1 and Appendix D.

2.4.1 Acute Toxicity

Acute toxicity data have been reported for oral, intravenous, and dermal studies.

2.4.1.1 Oral. Amitrole shows very low toxicity following ingestion of single doses. Weir et al. (1958) reported values for acute oral toxicity of amitrole ranging from 25,000 mg/kg for rats to 14,700 mg/kg for mice. Amitrole elicited no signs of toxicity when administered to rats by stomach tube at 4,080 mg/kg body weight (Gaines et al. 1973). In a review, Bailey and White (1965) reported an acute oral LD_{50} of 1,150 mg/kg for rats. This latter value appears to be inconsistant when placed in context with median lethal doses reported by others. For Amitrole-T®, however, the acute oral LD_{50} in rats has been reported as 5,000 mg/kg (Weir et al. 1958). A 39 year old woman showed no signs of intoxication following ingestion of amitrole at a dose of 20 mg/kg (Geldmacher-v. Mallinckrodt and Schmidt 1970).

2.4.1.2 Intravenous. No effect was observed when amitrole was injected intravenously into mice, rats, or dogs at dose levels of 1,600, 1,750, and 1,200 mg/kg, respectively (Weir et al. 1958). Amitrole injected intravenously into rabbits at 1,000 mg/kg resulted in decreased catalase activity in ocular tissues (Bhuyan et al. 1974).

2.4.1.3 Dermal. The LD_{50} for Amizol® applied to the shaven skin of rabbits was 10,000 mg/kg (U.S. Department of Agriculture 1981). A single dose of 1,500 mg amitrole/kg applied to the skin of rats resulted in no signs of toxicity.

2.4.1.4 Eye Irritation. Acute eye irritation studies in experimental animals have not been reported. Two gardeners, one accidentially exposed to amitrole and the other to Amizol®, were reported to have eye injuries (Swift 1976). No information on the exposure dose levels or severity of the eye injuries was presented.

2.4.2 Subchronic Toxicity

2.4.2.1 Oral. Subchronic toxicity studies show that amitrole affects primarily the thyroid gland. Rats fed amitrole in the diet at 15, 30, 60, or 120 ppm for two weeks exhibited thyroid gland enlargement in the 60 and 120 ppm groups (Jukes and Shaffer 1960). When rats ingested amitrole in the diet at 150 mg/kg/day for 90 days, a 15 percent loss of weight during the first 6 weeks of exposure was noted (Gaines et al. 1973). When administered in the diet at 1,000 and 10,000 ppm for 63 days, toxic signs consisted of altered body weight gains and fatty changes in liver cells (Weir et al. 1958). However, when administered in drinking water at 50 ppm (approximately 2.5 to 5 mg/kg) for 15 weeks, rats exhibited slight retardation of growth and enlargement of the thyroid (Weir et al. 1958). When mice were fed amitrole in the diet at a level of 1 percent (10,000 ppm), thyroid hyperplasia was induced. Whole body catalase activity was also inhibited (Feinstein et al. 1978a).

When Amizol® was administered in the diet or drinking water of rabbits at 0.2 percent concentration for 25 weeks, cataracts were produced. Earliest changes were seen after 2 to 4 weeks of Amizol® feeding (Bhuyan et al. 1973).

2.4.2.2 Intraperitoneal. Enlargement of the thyroid was observed in rats when the animals were treated with 21 doses of 1,000 mg/kg given intraperitoneally over a 45 day period (Weir et al. 1958).

2.4.3 Chronic Toxicity

In one study, rats fed amitrole in the diet at 50 ppm (2.5 mg/kg body weight) for 476 days showed no toxic effects. When rats were fed 100 ppm (5 mg/kg body weight), thyroid adenomas and adenocarcinomas were induced; however, when rats were fed amitrole in the diet at 500 ppm (25 mg/kg body weight) for 119 days, the thyroid glands were normal (U.S. Department of Agriculture 1981). In another study, rats were fed amitrole at dietary levels of

10, 50, 100, and 500 ppm (0.5, 2.5, 5, and 25 mg/kg body weight) for 2 years. At 13 weeks, male rats receiving 50 ppm (2.5 mg/kg body weight) exhibited enlarged thyroids. Female rats showed no effects. Levels of 150 and 500 ppm (5 and 25 mg/kg body weight) produced growth suppression, enlarged thyroids, and altered food utilization ratios. Microscopic examination of tissues revealed hyperplasia and toxic degeneration of the thyroids in rats on dietary levels of 50, 100, and 500 ppm amitrole.

2.4.4 Reproductive and Teratogenic Effects

In a multi-generation reproduction study, groups of male and female rats (designated as F_0) were fed 500 and 1,000 ppm amitrole for 107 to 110 days. Others were fed 25 and 100 ppm for 240 to 247 days. The progeny of these animals (designated as F_1) were fed 25 and 100 ppm amitrole for 141 days. Pups born to parents fed 500 and 1,000 ppm amitrole were smaller, and had atropic thymuses and spleens indicative of runting. There was no runting observed at lower dietary levels. Hyperplasia of the thyroid was observed in all animals fed 100 ppm or greater. In the group fed 25 ppm, 40 percent of the F_0 and F_1 females developed hyperplasia of the thyroid (Gaines et al. 1973).

When pregnant rats were treated with 20 or 100 mg amitrole/kg body weight by oral intubation from days 7 through 15 of gestation, no reproductive or teratogenic effects were observed. In another study (Tjalve 1974), pregnant mice were treated with 500, 1,000, 2,500, and 5,000 ppm amitrole in the drinking water from days 6 through 18 of gestation. No teratogenic effects were observed. Fetuses from dams treated with the three highest doses were small and underdeveloped with immature skeletons. Resorptions of embryos were high among the group treated with 5,000 ppm. No adverse effects were seen in animals treated with 500 ppm amitrole in the drinking water.

2.4.5 Carcinogenicity

Amitrole has been shown to be carcinogenic in animals. Individual studies are summarized in Appendix D. Induced tumors are generally restricted to the thyroid gland in rats and mice following oral administration of amitrole. Significant levels of benign and malignant liver tumors also have been observed in rats and mice. No tumors were detected in dogs following oral administration of amitrole.

Evidence suggests that amitrole may be carcinogenic for man. An epidemiologic study in Sweden of railroad workers exposed to herbicides containing amitrole and phenoxy acids showed an increase in stomach tumors over the number expected. No thyroid tumors were

observed in this study. The causal relationship between tumor induction and exposure to amitrole was unclear, since workers exposed to a combination of the herbicides were more severely affected than those exposed to amitrole alone. Some of the relevant studies are described below.

In an editorial concerning the hazard of amitrole residues on cranberries (DuShane 1959), it was reported that when rats were fed a diet containing 100 ppm amitrole for 2 years, a signifiant number of thyroid adenomas and adenocarcinomas were observed. Animals fed 50 ppm amitrole in the diet developed adenomas but no adenocarcinomas. In another study (Jukes and Shaffer 1960), no thyroid tumors were observed in rats fed amitrole in the diet at 500 ppm for 17 weeks. However, rats fed amitrole at 10, 50, and 100 ppm for 2 years developed thyroid adenomas (Jukes and Shaffer 1960 and Hodge et al. 1966).

When amitrole was administered in the drinking water at 20 to 25 mg/day or in the diet at 250 or 500 mg/day for their lifespan, rats developed thyroid tumors as well as malignant liver tumors (Napalkov 1969, cited in International Agency for Research on Cancer 1974). Rats were treated with amitrole in the drinking water at 2,500 ppm for 70 weeks. All rats developed goiters. Invasive tumors and adenomas of the thyroid were observed in a number of animals (Tsuda et al. 1976). The subcutaneous administration of amitrole at 125 mg/rat twice weekly for approximately 11 months resulted in the formation of tumors of the thyroid and liver. However, when 3 pellets of 500 mg amitrole were implanted under the skin of rats each month for 5 months, followed by the subcutaneous injection of 125 mg amitrole from the 6 through the 12th month, sarcomas developed at the sites of treatment in 50 percent of the animals. No thyroid or liver tumors were reported (Napalkov 1969, cited in International Agency for Research on Cancer 1974).

In a study designed to determine the potential carcinogenicity of a number of pesticides, amitrole was used as a positive carcinogen control (Innes et al. 1969). Mice were treated by stomach tube with 1,000 mg amitrole per kg body weight from 7 to 28 days of age. The animals were then treated with 2,192 ppm amitrole in the diet (equivalent to approximately 330 mg/kg body weight) for 53 to 60 weeks. Two hybrid strains of mice were used. Carcinomas of the thyroid were observed in 64 of 72 treated mice examined. No thyroid carcinomas were found in 338 control mice. Liver tumors (hepatomas) were observed in 67 of 72 treated mice and 14 of 338 control mice.

When either 0.1 or 10 mg amitrole was applied to the skin of mice, weekly for life or as a single injection of 10 mg amitrole per animal, no skin tumors or evidence of other tumors were observed

during an observation period lasting 39 to 82 weeks (Hodge et al. 1966). Amitrole was fed in the diet at a 1 percent concentration to strains of mice which were either normal or deficient in body catalase activity (acatalasemic). A larger portion of the acatalasemic mice developed liver tumors and the tumors were detected earlier than in normal mice. No thyroid tumors were reported (Feinstein et al. 1978b).

In life-time studies with rats, mice, and golden hamsters, the animals were fed diets containing amitrole at concentrations of 1, 10, and 100 ppm. At 100 ppm an increased number of thyroid and pituitary gland tumors were observed in rats. No tumor induction was observed in mice and golden hamsters (Steinhoff et al. 1983).

No tumors were detected when dogs were fed amitrole in the diet at 10, 50, 100, or 500 ppm (equivalent to 0.25, 1.25, 2.5, and 12.5 mg/kg body weight/day) for 52 weeks (Hodge et al. 1966).

The morphological transformation of mammalian cells in culture is an in vitro endpoint which reflects the potential carcinogenicity of chemicals. Amitrole induced morphological transformation in early passage Syrian hamster embryo cells (Pienta et al. 1977; Inoue et al. 1981; and Dunkel et al. 1981) following treatment of the cells with the chemical at levels of 0.1 to 100 ppm. Rat embryo cells, concurrently injected with a murine leukemia virus and subsequently treated with amitrole at dose levels of 10 to 1,200 ppm, also exhibited morphological transformation (Dunkel et al. 1981).

An epidemologic study of 348 Swedish railroad workers exposed to various herbicides, mainly amitrole and phenoxy acids such as 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T), during the period 1957-1976, indicated a possible relationship between exposure to the herbicides and an increased overall tumor morbidity and mortality (Axelson and Sundell 1974). In an updated study (Axelson et al. 1980), which followed the group of workers through 1978, the data were divided into subgroups with exposure to either amitrole, phenoxy acids, or to a combination of the two herbicides. This updated analysis of the causes of death among the railroad workers with exposure to the herbicides showed that the observed number of deaths was higher than expected, especially among those workers with an early exposure to a combination of both amitrole and phenoxy acids. The mortality among persons exposed to amitrole alone was lower than expected; however, three of the four deaths observed were attributed to tumors. No specific type of tumor was predominant, although there were three stomach tumors and three lung tumors among the variety of tumors reported. Instead, the trend seemed to be an increase in overall tumor mortality. The causal relationship of tumor production to

exposure to specific herbicides remains unclear, since workers exposed to a combination of amitrole and phenoxy acids seemed to be the most seriously affected.

2.5 Mutagenicity

Amitrole has been evaluated extensively in a variety of genotoxicity test systems designed to determine the extent to which the compound induces mutations or otherwise causes damage to DNA. Tests were performed in systems employing bacteria (Salmonella typhimurium, Escherichia coli, Bacillus subtilis, Streptomyces coelicolor), yeasts (Saccharomyces cerevisiae), fungi (Aspergillus nidulans), bacteriophage (E. coli), mammalian cells (HeLa, EUE, Chinese hamster ovary, human lymphocytes, mouse bone marrow, mouse embryo), and insects (fruit fly, Drosophila melanogaster). Results from the individual tests are summarized in Appendix E. Amitrole is considered to be nonmutagenic since negative results were obtained in the Ames Salmonella/microsome bacterial mutagenicity assay when tested at least 14 times with strain TA98, 13 times with strains TA100 and TA1535, 12 times with strain TA1537 and 4 and 6 times with strains TA1536 and TA1538 respectively. Negative mutagenicity results were also obtained in systems employing the fungus A. nidulans, E. coli bacteriophage, and Drosophila melanogaster. No evidence of sister chromatid exchanges were observed when amitrole was tested with Chinese hamster ovary cells. Amitrole did not produce chromosomal aberrations in human lymphocytes in culture or bone marrow cells of mice. Negative results were also obtained in a mouse cell system designed to measure an increase in infection by a murine leukemia virus. Since most of the other standard in vitro tests gave negative results, only the few giving positive responses are discussed here.

Simmon et al. (1979) reported that amitrole gave positive responses in a host-mediated mutagenicity assay employing S. typhimurium strains TA1530 and TA1538 in mice treated with 12 and 125, but not 250 mg amitrole/kg. A host-mediated assay employing S. typhimurium strain TA1950 was negative when mice were treated with 1450-2900 μmole of amitrole alone. When treated with equimolar combinations of amitrole and nitrite, positive results were obtained indicating that amitrole can be nitrosated to an active mutagenic compound (Braun et al. 1977). Kada (1981) reported the differential inhibition of a repair deficient strain of B. subtilis when tested in the presence of metabolic activation S-9 fractions prepared from yellowtail fish or clams. However, amitrole gave a negative response when tested in the presence of conventional S-9 fraction prepared from rat liver. Venitt and Crofton-Sleigh (1981) reported that amitrole was mutagenic for E. coli strain WP2uvrA, when tested at 10 to 500 μg/plate. Nine other studies with E. coli by other investigators gave negative results. Shirasu et al. (1981) reported that the

same strains of B. subtilis gave negative responses when tested with amitrole. By using a mutation system based on the induction of resistance to low levels of streptomycin in the filamentous bacterium Streptomyces coelicolor, Carere et al. (1976, 1978) and Carere and Morpugo (1981) showed that amitrole was weakly mutagenic when tested at 1,000 μg/plate. This novel forward mutation system has not been further evaluated. Sharp and Parry (1981a) observed an inhibition of DNA repair when repair deficient and wild type strains of S. cerevisiae were tested with amitrole at 100 to 750 ppm. These authors (Sharp and Parry 1981b and Parry and Sharp 1981) also reported that amitrole was mutagenic for S. cerevisiae strains JD1 and D6.

Amitrole appears to damage DNA as evidenced by positive responses for unscheduled DNA synthesis observed with HeLa cells (Martin and McDermid 1981) and EUE cells (Benigni and Dogliotti 1980a,b).

With the exception of the few positive studies, the overwhelming body of data were negative. Therefore, amitrole is considered not mutagenic.

3.0 Environmental Fate

Amitrole is an effective herbicide for a variety of annual and perennial grasses and broadleaf weeds, yet it has a low to moderate persistence in soil, appears generally to disappear fairly rapidly from water, and has a relatively low toxicity to most animal species. The effectiveness of amitrole is due largely to foliar uptake of the herbicide followed by translocation through the plant to other structures. Amitrole is readily metabolized in plants. In animals, amitrole is primarily excreted unchanged in urine although some is eliminated in feces and small amounts are metabolized (Carter 1975 and Ghassemi et al. 1981).

3.1 Bioaccumulation and Metabolism

3.1.1 Plants

The major pathways for uptake of amitrole in plants is through the leaves and roots. Amitrole uptake can be affected by variations in plant leaf structure which alter wetting of the leaf and subsequent herbicide penetration (Carter 1975).

More than a dozen compounds have been isolated from amitrole-treated plants and identified as derivatives of amitrole (Ashton 1963 in Ghassemi et al. 1981). A major metabolic breakdown product of amitrole is 3-(3-amino-s-triazole-1-yl)2-aminopropionic acid (3-ATAL). This metabolite appears to have a lower toxicity than the parent amitrole and to be less mobile. Thus the metabolism of amitrole to 3-ATAL is assumed to result in detoxification of the parent amitrole. Ammonium thiocyanate inhibits formation of 3-ATAL and is thus added to formulations such as Amitrol-T® and Cytrol Amitrole-T® to inhibit detoxification of the herbicide in the plant and to maintain amitrole mobility (Carter 1975).

There is some degree of variation in metabolism of amitrole in different plant structures and species. Miller and Hall (1961) identified a metabolite they designated as Y as the most abundant metabolite of amitrole in cotton leaves, whereas in the seed, they found 3-ATAL as the predominant metabolite. In such plants as beans, Canada thistle, bindweed, silver maple, honeysuckle, and alfalfa, however, 3-ATAL was identified as the primary metabolite of amitrole. Herrett and Bagley (1964) isolated a metabolite of amitrole from Canada thistle that they designated as Unknown III. This metabolite had a strong herbicidal activity and was 5 to 8 times as effective as the parent amitrole in suppressing growth of tomato and lettuce roots. The molecular structures of the metabolites other than 3-ATAL have not been determined.

3.1.2 Animals

In animals, amitrole is rapidly absorbed and eliminated, primarily in the urine, essentially as the parent compound but with small amounts of metabolites. Because amitrole is readily soluble in water but relatively insoluble in fats such as cellular lipids, it does not tend to accumulate in plant and animal tissue to any great extent. The major studies of bioaccumulation and metabolism of amitrole in animals are summarized in Appendix F.

Most of radiolabelled amitrole fed to rats in a single dose of 50 mg/kg body weight was excreted in the urine within 24 hours. Within three days, only 6 percent of the original dose had been metabolized to other compounds (Grunow et al. 1975).

Fang et al. (1964) fed radiolabelled amitrole to rats at a dose of 1 mg per rat and then analyzed expired air, urine, feces, internal organs, and tissues for radioactivity. After absorption, the parent radiolabelled amitrole was distributed throughout most of the body tissues but was rapidly eliminated. During the first 24 hours after ingestion, 70 to 95.5 percent of the radioactivity was found in the urine, primarily as parent amitrole along with two metabolites. In a second study, radiolabelled amitrole was fed to rats at doses from 1 to 200 mg per rat to determine whether the excretory pattern and tissue accumulation of amitrole, and metabolite formation, are a function of oral dose (Fang et al. 1966). No significant difference in the percent recovery of radioactivity in urine and feces was observed in relation to the administered dose.

A 39-year old woman ingested a commercial preparation of 30 percent amitrole (20 mg/kg body weight) and 56 percent diuron (3,4-dichlorophenyl N,N'-dimethylurea). Fifty percent of the estimated intake was found in the woman's urine within a few hours after ingestion as unchanged amitrole. No metabolites were identified and no signs of intoxication in the woman were observed (Geldmacher-v. Mallinckrodt and Schmidt 1970).

Franco and Municio (1975) compared the metabolism of amitrole in animals, plants, and bacteria. Unaltered amitrole and three metabolites were identified in the urine of treated rats. Unaltered amitrole appeared in the urine of the rats within 24 hours of initial treatment, and amitrole and metabolite excretion ceased within 24 hours of the final treatment.

3.2 Soil

In the soil, amitrole is adsorbed to colloids or is complexed with metallic ions such as nickel, cobalt, copper, iron, or magnesium

(Sund 1956 in Ghassemi et al. 1981). Amitrole has a very short persistence (1 month or less) in soil compared with other pesticides and thus exhibits only temporary effects on soil biota (Ashton 1982). Disappearance from soil has been attributed to adsorption and microbial degradation, although it appears that the primary means of amitrole degradation is by nonbiological reactions in which it is oxidized by free radicals. Because of the low volatility of amitrole, volatilization is not considered to be a major route for loss of amitrole from the soil. Amitrole appears to be stable under ultraviolet light, consequently photodecomposition in natural conditions does not appear to be an important degradation process for amitrole. In the laboratory, however, photodecomposition of amitrole has been demonstrated under ultraviolet light in the presence of riboflavin (Vitamin B_2), a common chemical in biological systems (Carter 1975 and Ghassemi et al. 1981).

Amitrole is adsorbed to soil particles fairly readily, but this adsorption is also readily reversed by water percolating through the soil (Ghassemi et al. 1981). Compared to other pesticides, amitrole is relatively mobile in silty clay loam soils (Helling 1971). The capacity of soils to adsorb amitrole is a function of organic matter content, acidity, and possibly base exchange capacity of the soil. In a high organic soil suspension of peat and water, more than 59 percent of applied amitrole was adsorbed onto soil particles within one minute and more than 68 percent was adsorbed within 24 hours (Nearpass 1969). In organic soil, amitrole adsorption was as high as 40 percent, whereas in an inorganic sandy soil, only 5 percent of applied amitrole was adsorbed onto soil particles (data cited in Ghassemi et al. 1981). Amitrole is more readily adsorbed in acidic soils than in basic soils (Nearpass 1969). Several authors have examined the adsorption of amitrole relative to the base exchange capacity of soils and have found generally that amitrole adsorbs rapidly and tightly to particles in soils with a high base exchange capacity, which would be expected in soils with large amounts of organic matter that can serve as a chelating agent and as a sorbent to bind up nonionic molecules such as amitrole.

Adsorption of amitrole to soil particles tends to decrease the availability of amitrole for leaching. As a result, amitrole is more readily leached from sandy soils in which there has been little adsorption than from high organic soils in which more amitrole is adsorbed. Amitrole is relatively highly soluble in water and consequently it moves readily with the leaching water.

Ghassemi et al. (1981) describe amitrole as having a low to moderate persistence in soil, depending on soil type and environmental conditions. Persistence of amitrole in soil is influenced by soil moisture, temperature, acidity, base exchange capacity, and activity of soil microorganisms. Studies by Ercegovich and Frear

(1965 in Ghassemi et al. 1981) indicated that amitrole persistence decreases with increasing soil moisture, probably as a result of an increased rate of degradation and/or leaching. Residues were also decreased as soil temperatures were raised to as much as 212°F (100°C). Loss of amitrole continuing at temperatures as high as 212°F (100°C) confirms that degradation is at least partially due to chemical processes, since microbial activity would not continue at such elevated temperatures. These authors also found that amitrole persistence was shortened by pH levels that were much above or below neutral (pH 7.0). Sund (1956 in Ghassemi et al. 1981) found that a high base exchange capacity in the soil increased persistence of amitrole while high clay and organic matter decreased persistence.

In studies of adsorption and desorption of amitrole on forest floor litter material (air dried red alder litter) in the laboratory (Norris 1970, 1971, and 1976 and Tarrant 1971), it was found that amitrole degrades rapidly. When amitrole was applied at a rate equivalent to 2 lb/acre (4.2 kg/ha), only 20 percent of the applied material remained adsorbed onto the litter after 35 days. Tarrant (1971) concluded that amitrole would not be available for transport through soil to streams within one summer after application.

Several authors have studied the interactions between microorganisms and amitrole applied to the soil, resulting in controversy over the relative role of soil microorganisms and nonbiological chemical processes in the degradation of amitrole (Carter 1975 and Ghassemi et al. 1981). In a study of the effects of amitrole and 3,5-D (a chemical derivative of amitrole) on nitrogen fixation by blue-green algae, DaSilva et al. (1975) found an initial depression in nitrogen fixation followed by increased activity. Several soil bacteria of the genera Pseudomonas, Bacillus, and Corynebacterium will degrade amitrole following a three-week lag period if sufficiently diluted (studies cited in Ghassemi et al. 1981). Laboratory studies of microbial degradation of amitrole should not, however, be taken to indicate that such degradation plays a major role in natural environments. Kaufman (1966) showed that amitrole inhibited microbial degradation of dalapon, another herbicide, but that dalapon did not inhibit microbial degradation of amitrole. Franco and Municio (1975) found that bacterial growth (Escherichia coli) was repressed with levels of 0.1 percent amitrole in the culture medium, and Bamford et al. 1976 were able to grow both E. coli and Salmonella typhimurium in complete culture media at amitrole concentrations of 1.7 to 2.4 percent. Cooper et al. (1978) found that 50 ppm of the amitrole formulation Weedazol-T® resulted in some reduction of culture growth for bacteria, actinomycetes, fungi, and yeasts. Fletcher (1960 in Ghassemi et al. 1981) found reduced nitrification rates and reduced microorganism populations following field applications of amitrole at rates ranging from 1 to 8 lb/acre (1.1 to 9.0 kg/ha). Other studies with amitrole have demonstrated

inhibition of microbial respiration and nitrification in activated sludge, inhibition of nitrifying bacteria in soil, and inhibition of cellulose decomposition by fungi (Ghassemi et al. 1981).

3.3 Water

The fate and persistence of amitrole in aquatic systems has not been studied to any great extent. The major studies that have been conducted, however, indicate that normal field applications of amitrole do not result in appreciable amounts of amitrole residues in stream systems. Furthermore, these studies indicate that any amitrole residues that do get into the stream systems are rapidly dissipated or adsorbed onto bottom sediments.

Valentine and Bingham (1976) demonstrated that algae of several species were able to metabolize amitrole and thereby remove the herbicide from water. From these studies, they concluded that accumulation of amitrole "would not appear to be a problem."

Amitrole concentrations were monitored in downstream creek waters from a small, 100-acre watershed that had been aerially sprayed with amitrole at a rate of 2 lbs/acre (2.2 kg/ha) for salmonberry control (Marston et al. 1968). Amitrole was found in measurable amounts in water samples near the downstream edge of the sprayed area during the spray operation and for 5 days subsequent to spray. The maximum amitrole concentration observed was 155 ppb (parts per billion), found in a sample 30 minutes after initiation of spray. By the end of the 2-hour spray operation, amitrole levels in the streamflow had decreased to 26 ppb, and six days after spray there were no detectable levels of amitrole in the streamflow. By the end of the study, only 15.19 grams, representing 0.0167 percent of the 200 lbs. of the total active ingredient applied to the watershed, were calculated to have left the area in the 457,000 gallons of streamflow leaving the area over the six-day post-spray period. It should also be noted that 1.8 miles below the sprayed area, amitrole concentrations were below detectable levels, due probably to a combination of dilution and both degradation and adsorption in the stream bottom.

Norris (1968) reported on a study of amitrole residues in streams flowing nearby or through forest areas treated with amitrole. Creek water flowing through an area treated in March was sampled during the following September and October (average daily rainfall of 0.63 inches) and did not contain measurable quantities of amitrole. As pointed out by Norris and Moore (1970), quantities of herbicide that can be found in streams are a function of the location of the spray unit boundaries with respect to the stream. Highest concentrations of herbicides are found in streams originating in or flowing

through the spray zone, lowest concentrations in streams totally excluded from the spray zone.

Amitrole residues and dissipation rates in irrigation water were examined by Demint et al. (1970) since amitrole is often used for the control of ditchbank vegetation. Following application of Amitrol-T® at rates of 4 and 3 lb/acre (4.5 and 3.4 kg/ha) to a single ditchbank of 2 flowing canals, maximum concentrations of 31 and 43 ppb amitrole were measured in the canal flow. When two banks of a dry canal were treated with Amitrol-T® at a rate of 6 lb/acre (6.7 kg/ha) and water was introduced after 6 days, a maximum of 98 ppm amitrole was obtained in the flow. In these canals, amitrole levels had decreased to about 1 ppm 2 hours after passage of the main body of amitrole-bearing water by the last sampling station. The authors found that the dissipation of amitrole in the canal waters, for distances up to 8 miles (14.5 km), was a linear function of amitrole concentration and downstream flow.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to amitrole during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved amitrole. Calculations of exposure to amitrole in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to amitrole are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Amitrole Application

Available application data for amitrole use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to amitrole differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of amitrole in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Amitrole is adsorbed to colloids or is complexed with metals in soil. Its persistence in soil is among the shortest (1 month) of the pesticides and is a function of soil pH, moisture, temperature and texture. However, amitrole is soluble in water and will persist more than 200 days in aquatic conditions although it tends to adsorb readily to sediments. The potential for incidental exposure of humans and wildlife to amitrole is low, existing only for a short period after its application.

4.2 Exposure

Estimates of potential exposure to amitrole are based on aerial as well as ground application. The maximum aerial application rate for amitrole (see Table 1-1) is 1.8 lb a.i./acre, while the compound is applied from the ground (broadcast) at rates as high as 5.5 lb a.i./acre. In both aerial and ground application, amitrole is applied

Table 4-1

USDA Forest Service Application Data for Amitrole (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Amitrole (14)	Ground Foliar	4	56	4.5	5.3	5.9	8.1	Noxious Weeds (6) Recreation (4) Range (2) Wildlife (1) Timber (1)

Source: Gross 1983

as a liquid. The calculations in Table 4-2 that are based on aerial application (e.g., spray/observer dose, wildlife dermal exposures, and water concentrations) assume an application rate of 1.8 lb a.i./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose, and wildlife oral exposures) assume an application rate of 5.5 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of amitrole and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of amitrole to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational exposure estimates for amitrole are based on exposures on a per pound per acre application rate multiplied by 1.8 lb/acre for observers and 5.5 lb/acre for backpack sprayers. In the case of the aerial spray observer, dose estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. Since amitrole has a low solubility in organic solvents other than ethanol, this is a conservative estimate for dermal absorption. At 1.8 lb a.i./acre aerial application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.072 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 5.5 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.22 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Amitrole

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader Observer Backpack Sprayer	0.1 mg/kg up to 0.072 mg/kg 0.22 mg/kg		[b]
Environmental Exposure			
Fish	0.018 ppm (Immersion)		[c]
Rabbits	0.725 mg/kg	Negligible	5.78 mg/kg
Deer	0.182 mg/kg	Negligible	7.92 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.00036 mg/kg (2 days)
(Rabbits)	NA	NA	0.025 mg/kg (2 days)
(Deer)	NA	NA	0.032 mg/kg (2 days)
(Water)	NA	NA	0.00072 mg/kg (1 day)
(Berries)	NA	NA	0.0018 mg/kg (1 day)
(Mushrooms)	NA	NA	0.00090 mg/kg (1 day)
Toxicity Summary	Acute LD_{50} = 10,000 mg/kg in rabbits	NA	Acute oral LD_{50}'s >1,000 mg/kg in rats and mice. Chronic feeding of 100 ppm in rats caused thyroid adenomas and adenocarcinomas.
	Most studies, LC_{50} >100 ppm for fish		

[a]Assumes aerial application at 1.8 lb active ingredient per acre and ground foliar application at 4 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure.

[c]Fish exposure results from immersion in water containing amitrole.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation expousre. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by amitrole application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 1.8 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 0.7254 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.1818 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of amitrole vapor would be negligible since the vapor pressure of amitrole is reported to be low (Carter 1975). Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of amitrole in water, calculated as:

Water concentration: 0.018 ppm (0.01 ppm at 1.0 lb/acre aerial application rate)

This concentration of amitrole represents the estimated maximum concentration immediately after spraying. Although amitrole can potentially be leached from soil and carried into waterbodies in runoff water, monitoring studies indicate very low concentrations of amitrole residues in ditches after ditchbank application of amitrole (Demint et al. 1970).

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of amitrole by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 5.5 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 7.920 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 5.775 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of amitrole in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.018 ppm

- bioconcentration: 1.0

Fish tissue concentration = 0.018 ppm (0.018 mg/kg)

Deer and rabbit tissue concentrations include amitrole accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied amitrole is absorbed

at a rate of 10 percent for reasons discussed in Section 4.2.1 with regard to spray observer absorption rates. It is also assumed that oral exposure of deer and rabbit to amitrole lasts only for 2 days due to the relatively short environmental persistence of amitrole and that 10 percent of the ingested amitrole is assimilated. In view of the rapid (on the order of 2 to 3 hour half-life) elimination of amitrole from mammals indicated in Appendix F, this latter assumption is a conservative one. Deer tissue concentrations of amitrole are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.1818 mg/kg (dermal exposure) x 0.10 = 0.01818 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 7.92 mg/kg/day (oral exposure) x 0.10 x 2 days = 1.584 mg/kg.

Total Deer Tissue concentration = 0.01818 mg/kg + 1.584 mg/kg = 1.6022 mg/kg (1.602 ppm).

Rabbit tissue concentrations of amitrole are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.7254 mg/kg (dermal exposure) x 0.10 = 0.07254 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation absorption of two-days feeding, or 5.775 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.155 mg/kg.

Total Rabbit Tissue Concentration = 0.07254 mg/kg + 1.155 mg/kg 1.2275 mg/kg (1.228 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to amitrole from eating meat are derived:

Fish meat: 0.018 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.00036 mg/kg

Deer meat: 1.602 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.032 mg/kg

Rabbit meat: 1.228 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.025 mg/kg

Water and plant consumption by man would result in the following oral exposures to amtirole based on assumptions stated in the General Introduction of the Herbicide Background Statements at an application rate of 1.8 lb a.i./acre (aerial):

Water: 0.00072 mg/kg (0.0004 mg/kg at 1 lb/acre application)

Berries: 0.0018 mg/kg (0.001 mg/kg at 1 lb/acre application)

Mushrooms: 0.00090 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of amitrole to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rodent acute oral LD_{50}'s, and immersion LC_{50}'s for fish).

As part of its reregistration guidance for pesticide products containing amitrole, the U.S. Environmental Protection Agency (1984) provided preliminary exposure estimates for various applications of amitrole. The maximum daily exposures incurred during forest application were estimated to be 0.05, 0.30, 0.004, and 3.0 mg/kg for knapsack applicators, knapsack mixer/loaders, helicopter pilots, and helicopter mixer/loaders, respectively, and concluded that dermal exposures through the hands was the major route of exposure. These maximum exposure estimates for knapsack applicators and helicopter pilots are less than the dose estimates for applicators (backpack sprayers) and observers used in this assessment. Mixer/loader exposure estimates are higher than dose estimates made here, but with the assumption of a 10 percent absorption of dermal exposures, the maximum exposure for mixer/loaders during helicopter application (3.0 mg/kg/day) becomes a dose of 0.3 mg/kg/day, slightly more than the estimated dose (0.22 mg/kg) for backpack sprayers in this assessment.

Small mammal studies have shown that amitrole applied to the skin of rabbits does not cause toxic signs. Eye injuries have been reported in two gardners accidentally exposed to amitrole. Rats fed 100 ppm amitrole for 2 years developed thyroid adenomas and adenocarcinomas, while the lower dose of 50 ppm amitrole in the diet only induced adenomas. Thyroid hyperplasmia and runting of progeny, but no apparent teratogenic effects, have been observed in rats fed 25 to 100 ppm amitrole. Although nonmutagenic in the Salmonella typhimurium bacterial system and most other systems tested, amitrole has been shown to induce DNA damage. These studies are summarized in

Table 2-1 and detailed in the Appendices. Generally, they suggest that amitrole may have the potential to induce adverse reproductive or carcinogenic effects.

Although there is sufficient evidence that amitrole is carcinogenic in animals, there are insufficient epidemiological data concerning its carcinogenicity in humans. Railroad workers exposed to amitrole and other herbicides had a slight excess of cancer when all sites were considered together. Whether the excess cancer was to amitrole alone is unknown. For forestry applications, the extremely small exposures calculated here and their short duration (8.1 days per year and 5.9 hours per day) are orders of magnitude less than those that have been shown to cause cancer in laboratory animals.

Appendix A

Summary of Amitrole Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Amitrole (50% a.i. formulation)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	24 hours 48 hours 96 hours	IC_{50} = 53.50 ppm IC_{50} = 3.55 ppm IC_{50} = 1.43 ppm	Some sublethal effects were noted, including retarded growth, inhibited reproduction, reduced hatching success.	Findley 1966 in Bunting and Robertson 1975
Amitrole	Copepod (Cyclops vernalis; Crustacea, 0-4 hour nauplii larvae).	Surface absorption, static bioassay	48 hours 96 hours	LD_{50} = 58.5 ppm (39.76 to 124.0)[a] LD_{50} = 22.1 ppm (18.54 to 27.71)	pH = 6.7	Bunting and Robertson 1975, and Robertson and Bunting 1976
Amitrole	Honeybees (Apis mellifera; Insecta)	Surface absorption	--[b]	Mortality less than 5% when bees dusted at 12.09 g/bee	--	Atkins et al. 1975 in Ghassemi et al. 1981
Amitrole	German cockroach (Blattella germanica; Insecta)	Ingestion	--	Amitrole at 1,000 ppm in food was toxic for nymphs (100% mortality) and reduced adult fecundity.	--	Riviere 1976
Amitrole	Fruit fly (Drosophila melanogaster; Insecta)	Surface absorption	Through larval development	LD_{50} = 40 ppm in medium	Development time prolonged at concentrations as low as 10 ppm.	Laamanen et al. 1976

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Amitrole	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption	up to 24 hours	Animals became immobile. Molting individuals were more rapidly immobilized.	Kept at 24°C (75°F)	Schultz and Kennedy 1976
Amitrole	Cladoceran (Daphnia magna; Crustacea)	Surface absorption	26 hours	Median immobilization concentration = 23 ppm	--	Crosby and Tucker 1966 in Bunting and Robertson 1975
Amitrole	Nematode (Acrobeloides buetschlii)	Surface absorption	--	50% mortality at 184 ppm, almost total mortality at 600 ppm	At 300 ppm, egg laying was inhibited with tendency for larvae to hatch within adult. Cuticular outgrowths were common.	Frey 1979
Amitrole	Honeybees (Apis mellifera; Insecta)	Ingestion	6 weeks	250 ppm in sugar syrup fed to bee colonies did not result in changes in colony weight compared to controls. No abnormalities attributable to amitrole were observed.	--	King 1964
Amitrole	Brine Shrimp (Artemia sp.; Crustacea)	Surface absorption, static bioassay	up to 36± 5 days	Reduced zygote production when tested in 5 ppm amitrol in water.	--	Grosch 1980
Cytrol Amitrole-T®	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} 18.0 to 56.0 ppm	21°C (70°F), first instar.	Johnson and Finley 1980

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Cytrol Amitrole-T®	Isopod (Asellus) sp.; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	15°C (59°F), mature animals	Johnson and Finley 1980
Amitrol-T®	Copepod (Cyclops vernalis; Crustacea 0-4 hour nauplii larvae)	Surface absorption, static bioassay	48 hours 96 hours	LD_{50} = 0.49 ppm (a.i.) (0.38 to 0.81) LD_{50} = 0.43 ppm (a.i.) (0.40 to 0.51)	pH = 6.7	Bunting and Robertson 1975
Amitrol-T®	Cladoceran (Daphnia magna; Crustacea)	Surface absorption	48 hours	LD_{50} = 40 ppm	--	Crosby and Tucker 1966 in Bunting and Robertson 1975
Amitrol-T®	Waterflea (Daphnia magna; Crustacea)	Suface absorption, static bioassay	48 hours	TL_{50} = 30.0 ppm	21.0°C (70°F), pH 7.4	Sanders 1970
	Seed shrimp (Cypridopsis vidua; Crustacea)			TL_{50} = 32.0 ppm	21.0°C (70°F), pH 7.4	
	Scud (Gammarus fasciatus; Crustacea)			TL_{50} >100.0 ppm	15.5°C (60°F), pH 7.4	
Amitrol-T®	Sowbug (Asellus brevicaudus; Crustacea)			TL_{50} >100.0 ppm	15.5°C (60°F), pH 7.4	
	Glass shrimp (Palaemonetes kadiakensis; Crustacea)			TL_{50} >100.0 ppm	21.0°C (70°F), pH 7.4	
	Crayfish (Orconectes nails; Crustacea)			TL_{50} >100.0 ppm	15.5°C (60°F), pH 7.4	
Amitrole	Nematodes	Field application at 5 lb/acre (5.6 kg/ha)	Not given	Forty-nine percent reduction in number of nematodes.	--	Courtney et al. 1962 in Pimentel 1971

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Amitrole	Bacterium (Escherichia coli)	Surface absorption	Cultured to 100 hours	Bacterial growth repressed by amitrole at 0.1% in culture medium.	Growth-curve lag phase prolonged and cell growth (numbers) below control. Effect of amitrole was partially reversed with addition of 0.1% adenine and 0.1% histidine to medium.	Franco and Municio 1975
Amitrole	Bacteria (Escherichia coli and Salmonella typhimurium)	Surface absorption	24 hours	In minimal medium, growth was repressed at 0.1% concentration and inhibited at 0.5%. In complete medium, bacteria tolerated concentrations of 1.7 to 2.4%.	--	Bamford et al. 1976
Weedazol-T®	Bacteria	Surface absorption	7 days	Nine percent of 50 cultures showed reduced or no growth at 50 ppm herbicide in medium.	--	Cooper et al. 1978
Weedazol-T®	Actinomycetes	Surface absorption	7 days	Two percent of 40 cultures showed reduced or no growth at 50 ppm herbicide in medium.	--	
Weedazol-T®	Fungi	Surface absorption	7 days	Twenty-seven percent of 70 cultures showed reduced or no growth at 50 ppm herbicide in medium.	--	
Weedazol-T®	Yeasts	Surface absorption	7 days	Seven percent of 15 cultures showed reduced or no growth at 50 ppm herbicide in medium.	--	Cooper et al. 1978

[a]95 percent confidence limits in parentheses.
[b]Data not available or not applicable.

Appendix B

Summary of Amitrole Toxicity Data for Fish and Amphibians

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
			FISH			
Amitrole	Rainbow trout	Oral: feeding at 1,200 and 4,800 ppm in diet	9, 12, 15, 16 and 20 months	High incidence (24 and 56%) of hepatomas at 9 months, low incidence (6 to 21%) at 12, 16 and 20 months, and none at 15 months. 1,200 ppm and 4,800 ppm in diet.	No pattern of correlation in hepatoma incidence with either dose or duration. Hepatoma incidence in controls did not exceed 1%. Diet composition and possible contamination were not indicated.	Halver 1967 in International Agency for Research on Cancer 1974
Amitrole	Largemouth bass	Static bioassay	--[a]	Nontoxic at 1,000 ppm	--	Bond et al. 1959 in Lorz et al. 1979
		Flow-through bioassay	6 days	All fish died at 1,000 ppm	--	
Amitrole	Coho salmon	Flow-through bioassay	48 hours	LD_{50} = 325 ppm	Water pH 7.5 to 7.7. Total alkalinity 41 to 71 ppm	
Amitrole	Mosquitofish	Static bioassay	96 hours	LC_{50} >10 ppm	18°C	Johnson and Finley 1980
Amitrole	Fathead minnow	Static bioassay	96 hours	LC_{50} >100 ppm	18°C	
Amitrole	Channel catfish	Static bioassay	96 hours	LC_{50} >160 ppm	18°C	
Amitrole	Mugil cephalus (Common name not given)	Static bioassay	24 hours 48 hours 72 hours 96 hours	LC_{50} = 320 ppm LC_{50} = 180 ppm LC_{50} = 100 ppm LC_{50} = 68.0 ppm	--	Tag El-din et al. 1981

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
			FISH (continued)			
Amitrole	Salmon	--	48 hours	Estimated LC_{50} = 3,250 ppm	--	Bohmont 1976 in Pimentel 1971.
Amitrole	Bluegill	Static bioassay	8 days	Survived 50 ppm	Fertilized eggs and fry.	Hiltibran 1967 in Lorz et al. 1979 and Pimental 1971
Amitrole	Green sunfish	Static bioassay	8 days	Survived 50 ppm	Fertilized eggs and fry.	Hiltibran 1967 in Lorz et al. 1979 and Pimental 1971
Amitrole	Lake chub-sucker	Static bioassay	8 days	Survived 50 ppm	Fertilized eggs and fry.	Hiltibran 1967 in Lorz et al. 1979 and Pimental 1971
Amitrole	Smallmouth bass	Static bioassay	8 days	Survived 50 ppm	Fertilized eggs and fry.	Hiltibran 1967 in Lorz et al. 1979 and Pimental 1971
Amitrole	Bluegill	--	24 hours	LC_{50} = 1,200 ppm	pH of 6.9; hardness of 29 ppm	Hughes and Davis in Lorz et al. 1979
Amitrol-T®	Mosquito fish	Static bioassay	24 hours 48 hours 96 hours	TL_{50} = 5,000 ppm TL_{50} = 3,100 ppm TL_{50} = 2,100 ppm	21 to 22°C	Johnson 1978
Amitrol-T®	Bluegill	Static bioassay	48 hours	Survived concentrations 100 ppm	24°C, pH 7.4	Sanders 1970
Amitrol-T®	Coho salmon (yearling)	Static bioassay	96 hours	LC_{50} = 70 ppm	A dose-dependent mortality was observed upon transfer to [illegible] water 24 to 48 hours after transfer. Mortality was not considered be due to osmoregulatory impairment.	Lorz et al. 1979

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
			FISH (concluded)			
Cytrol Amitrole-T®	Bluegill	--	96 hours	LC_{50} = 10,000 ppm	--	Cope 1963 in Pimentel 1971
Cytrol Amitrole-T®	Lake Emerald shiner	--	24 hours	LC_{50} = 455 ppm	Medium-hard water.	Swabey and Schenk 1963 in Pimentel 1971
Cytrol Amitrole-T®	Channel catfish	Static bioassay	96 hours	LC_{50} >160 ppm	18°C, 1.8 grams fish	Johnson and Finley 1980
Cytrol Amitrole-T®	Bluegill	Static bioassay	96 hours	LC_{50} >280 ppm	18°C, 1.1 grams fish	Johnson and Finley 1980
Ammonium Thiocyanate (ingredient in Amitrol-T® and Cytrol Amitrole-T®)	"Fish" (species not specified)	Not given	Not given	200 ppm lethal	--	Russian study reported by Springer 1957 in Pimentel 1971
			AMPHIBIANS			
Weedazol TL® Plus	Anuran (frog); Adelotus brevis (Amphibia, tadpoles common name not given)	Static bioassay	24 hours 48 hours 96 hours	TL_{50} = 5,200 ppm TL_{50} = 5,000 ppm TL_{50} = 3,000 ppm	Conducted at 21 to 22°C. Tadpoles subjected to subacute (1,000 ppm) dose for 96 hours had a reduced temperature tolerance compared to controls.	Johnson 1976

[a]Data not available or not applicable.

Appendix C

Summary of Amitrole Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Amitrole	White leghorn chick	Injected intraperitoneally with 500 mg/kg or 1,000 mg/kg body weight.	Varied from 3 to 38 days.	Reduced rate of weight gain and changes in microanatomy of thyroid gland.	--[a]	Wishe et al. 1979
Amitrole	Japanese quail	Ingestion	5 days	LC_{50} >5,000 ppm in diet	No mortality to 5,000 ppm. Birds were 12 days old at start of test.	Hill et al. 1975
Amitrole	Ring-necked pheasant	Ingestion	5 days	LC_{50} >5,000 ppm in diet	No mortality to 5,000 ppm. Birds were 10 days old of start of test.	
Amitrole	Mallard duck	Ingestion	5 days	LC_{50} >5,000 ppm in diet	No mortality to 5,000 ppm. Birds were 10 days old of start of test.	
Amitrole	Mallard duck	Ingestion (acute)	Observed 14 days after single dose	LD_{50} >2,000 mg/kg	Male birds, 3 to 4 months old. Loss of coordination, slight wing drop, and weakness was observed the first 3 days after dose.	Tucker and Crabtree 1970

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Amitrole	Mallard duck	Ingestion	Not given	Depressed reproduction in ducks fed doses 25% below levels that would result in mortality.	--	U.S. Department of the Interior 1962 in Pimentel 1971
Amitrole	Chicken (eggs)	Injected into eggs	Not given	Lowest concentration at which toxic effects were noted was 100 ppm.	--	Dunachie and Fletcher 1970
Amitrole	Chicken eggs	Aqueous solutions injected into yolk sacs at 0, 24, 48, and 96 hours of incubation. Up to 40 mg/egg.	Embryos examined after 19 days of incubation	Amitrole toxicity was dosage dependent and high at 0 and 96 hours, lower at 24 and 48 hours.	Principal malformations involved beak. Some malformations of the tibia were also observed.	Landauer et al. 1971

[a]Data not available or not applicable.

Appendix D

Summary of Amitrole Toxicity in Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Amitrole	Rat	Ingestion of a single dose	LD_{50} was 1,100 mg/kg	--[a]	Bailey and White 1965
Amitrole	Rat	Ingestion of a single dose	LD_{50} was 25,000 mg/kg	--	Weir et al. 1958
Amitrole	Rat	Stomach tube	No signs of toxicity with up to 4,080 mg/kg	--	Gaines et al. 1973
Amitrole	Mouse	Ingestion of a single dose	LD_{50} was 14,700 mg/kg	--	Weir et al. 1958
Amitrole	Human	20 mg/kg of amitrole ingested by a 39-year old woman	No signs of intoxication	The commercial preparation ingested contained 30% amitrole and 56% Diuron.	Geldmacher-v. Mallinckrodt and Schmidt 1970
Amitrol-T®	Rat	Ingestion of a single dose	LD_{50} was 5,000 mg/kg	--	Weir et al. 1958
			Acute Intravenous Toxicity		
Amitrole	Mouse	Intravenous injection	No effect at 1,600 mg/kg	--	Weir et al. 1958
	Cat	Intravenous injection	No effect at 1,750 mg/kg	--	
	Dog	Intravenous injection	No effect at 1,200 mg/kg	--	
	Rabbit	1,000 mg/kg intravenously	Decreased catalase activity in ocular tissues injected with 1,000 mg/kg	--	Bhuyan et al. 1974

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity		
Amitrole	Rat	Single dose applied to the skin	No signs of toxicity at 2,500 mg/kg	Hair was clipped.	Gaines et al. 1973
Amizol®	Rabbit	Applied to shaven skin	LD_{50} was 10,000 mg/kg	--	U.S. Department of Agriculture 1981
			Eye Irritation		
Amitrol	Human	Accidental exposure to a gardner	Eye injury reported	No information on exposure dose level or severity of eye injury.	Swift 1976
Amizol®	Human	Accidental exposure to a gardner	Eye injury reported	No information on exposure dose level or severity of eye injury.	Swift 1976
			Subchronic Oral Toxicity		
Amitrole	Rat	63-day oral administration of 1000 and 10,000 ppm in the diet	Altered body weight gain and fatty changes in liver cells	--	Cited in Weir et al. 1958
Amitrole	Rat	90-day, 150 mg/kg in diet	15% weight loss during first 6 weeks of exposure	--	Gaines et al. 1973
Amitrole	Rat	Fed in diet at 15, 30, 60, or 120 ppm for 2 weeks	Thyroid gland enlargement in 60 and 120 ppm groups	--	Jukes and Shafer 1960

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Concluded)		
Amitrole	Rat	50 ppm in drinking water for 15 weeks	Slight retardation of growth; thyroid enlargement	--	Cited in Weir et al. 1958
Amitrole	Rat	21 doses of 1,000 mg/kg given intraperitoneally over a 45-day period	Thyroid enlargement	--	Cited in Weir et al. 1958
Amitrole	Mouse	1% in diet for 12 weeks	Thyroid hyperplasia. Inhibition of whole body catalase enzyme activity	--	Feinstein et al. 1978
Amizol® (94.1% active ingredient)	Rabbit	Fed in diet or drinking water at 0.2% concentration for about 25 weeks	Cataract production. Earliest changes seen after 2-4 weeks of Amizol feeding. Inhibition of lens catalase enzymes	--	Bhuyan et al. 1973
			Chronic Toxicity		
Amitrole	Rat	Fed in diet at 10, 50, 100 and 500 ppm for 2 years	At 13 weeks levels up to 50 ppm had no effect on female animals; male animals had enlarged thyroid glands. Levels of 100 and 500 ppm produced suppression of growth, enlarged thyroids and altered food utilization. Hyperplasia and toxic degeneration of thyroids occurred at all dose levels.	--	Weir et al. 1958

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity (Concluded)		
Amitrole	Rat	Fed in diet at 50 ppm for 476 days	No toxic effects reported.	--	U.S. Department of Agriculture 1981
Amitrole	Rat	Fed in diet at 500 ppm for 119 days	Normal thyroid glands.	--	U.S. Department of Agriculture 1981
			Reproductive and Teratogenicity		
Amitrole	Rat	Multi-generation study. Male and female F_0 animals fed 500 and 1,000 ppm for 107-110 days, or 25 and 100 ppm for 240-247 days; F_1 animals fed 25 and 100 ppm for 141 days.	Pups born to parents fed 500 and 1000 ppm amitrole were smaller, and had atropic thymuses and spleens indicative of runting. No effect at lower dietary levels. Hyperplasia of thyroid in all animals fed 100 ppm or greater. In the 25 ppm groups, 40% of F_0 and F_1 females and 60% of F_1 males developed hyperplasia of the thyroid.	--	Gaines et al. 1973
Amitrole	Rat	Pregnant animals treated with 20 or 100 mg/kg of amitrole by oral intubation from days 7 through 15 of gestation.	No reproductive or teratogenic effects observed.	--	Gaines et al. 1973
^{14}C-labeled Aminotriazole	Mouse	Pregnant animals treated with 500, 1,000, 2500, or 5,000 ppm in drinking water from days 6 through 18 of gestation.	No teratogenic effects observed. Fetuses from dams treated with 3 highest doses were small and underdeveloped, with immature skeleton. Resorptions high among group treated with 5,000 ppm. No effect seen at 500 ppm.	--	Tjalve 1974

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity		
Amitrole	Rat	Fed in diet at 10, 50, and 100 ppm for 2 years	Thyroid adenomas developed in 1 of 10, 2 of 15, and 17 of 26 rats treated at the respective dose levels. No tumors in control animals.	--	Jukes and Shafer 1960
Amitrole	Rat	Fed in diet at 500 ppm for 17 weeks	No thyroid tumors.	--	Jukes and Shafer 1960
Amitrole	Rat	Fed in diet at 10, 50, and 100 ppm for 2 years	Thyroid tumors developed in 15 of 27 rats treated with 100 ppm amitrole. Incidence was 1-3 of 27 in groups treated with 10-50 ppm.	--	Hodge et al. 1966
Amitrole	Rat	20-25 mg/day in drinking water; 250 or 500 mg/day in diet for lifespan of the animals (10-32 months)	Thyroid tumors developed in 54 of 104 animals. Malignant tumors included hepatocellular and hepatocholangiocellular carcinomas in 5 of 44 rats receiving amitrole in drinking water; in 17 of 52 rats receiving amitrole in diet.	Occurrence of tumors by specific dose level not reported. No control data presented.	Napalkov 1969, cited in International Agency for Research on Cancer 1974
Amitrole	Rat	Subcutaneous administration at 125 ppm rat, twice weekly for approximately 11 months	5 thyroid tumors and 5 liver tumors developed in 7 animals	No information regarding controls.	Napalkov 1969, cited in International Agency for Research on Cancer 1974

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Continued)		
Amitrole	Rat	Implantation of 3 pellets of 500 mg amitrole under skin each month for 5 months. From 6th to 12th month 125 mg amitrole injected twice weekly subcutaneously	In 7 of 14 animals surviving at least 21 months 7 sarcomas developed at sites of treatment.	--	Napalkov 1969, cited in International Agency for Research on Cancer 1974
Amitrole	Rat	2,500 ppm in drinking water for 70 weeks	Goiters developed in all rats. Increased incidences of invasive tumors or papillary adenomas of the thyroid were observed.	Animals were either normal or had either partial thyroidectomies or heterotopic autoimplantation of thyroid tissue.	Tsuda et al. 1976
Amitrole	Rat	100 ppm in diet for 2 years	Significant number of thyroid adenomas and adenocarcinomas observed.	Summary statement only.	Cited in DuShane 1959
Amitrole	Rat	50 ppm in diet for 2 years	Animals developed adenomas but no adenocarcinomas.	Summary statement only.	Cited in DuShane 1959
Amitrole	Rat	Daily oral doses for unspecified time	Doses of 0.5 to 2,000 mg/kg lead to tumor formation.	Summary statement only.	Terracini 1971
Amitrole	Rat	Fed in diet at 100 ppm for 730 days	Thyroid adenomas and adenocarcinomas.	--	U.S. Department of Agriculture 1981
Amitrole	Mouse	1% Amitrole fed in diet to normal and acatalasemic mice	A larger portion of acatalasemic C3H mice developed liver tumors that were detected earlier than normal mice.	C3H and C57BC/6 mice strains were used.	Feinstein et al. 1978
Amitrole	Mouse	0.1 or 10 mg applied to skin weekly for life	No skin tumors observed.	--	Hodge et al. 1966

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Continued)		
Amitrole	Mouse	Single subcutaneous injection of 10 mg amitrole per animal	No evidence of carcinogenicity throughout 39-82 weeks observation period.	—	Hodge et al. 1966
Amitrole	Mouse	Mice treated by stomach tube with 1,000 mg/kg from 7 to 28 days of age. Then treated with 2,192 ppm (approximately 330 mg/kg) in diet for 53-60 weeks	Carcinoma of thyroid observed in 64 of 72 treated mice. No thyroid carcinomas in 338 control mice. Liver tumors (hepatomas) observed in 67 of 72 mice treated with amitrole and in 14 of 338 control mice.	Two hybrid strains of mice used, i.e. (C57 Bl/6 x C3H/A nf) F_1 and (C57 Bl/6 x AKR) F_1.	Innes et al. 1969
Amitrole	Mouse	Daily oral doses	Doses of 330 to 1,000 mg/kg lead to tumor formation.	Summary statement only.	Terracini 1971
Amitrole	Dog	Fed in diet at 10, 50, 100, or 500 ppm for 52 weeks	No tumors detected.	--	Quoted in Hodge et al. 1966
Amitrole	Human	348 Swedish railroad workers were exposed to herbicides during period of 1957-1972	There was an increase in overall tumor morbidity and mortality. The observed number of deaths was higher than expected, especially among those with an early exposure to both amitrole and phenoxy acids. The mortality among workers exposed to amitrole alone was lower than expected, however, 3 of the 4 deaths reported were due to tumors.	The causal relationship is unclear since the workers exposed to a combination of amitrole and phenoxy acids were more seriously affected.	Axelson and Sundell 1974 and Axelson et al. 1980

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Concluded)		
Amitrole	Rat cells	Cell cultures treated with 10, 80, 100, or 1,200 ppm.	Transformation of Rauscher-murine leukemia virus-infected rat embryo cells at 3 highest doses.	In vitro transformation.	Dunkel et al. 1981
Amitrole	Hamster cells	Cell cultures treated with 0.1, 1, 10, and 100 ppm.	Transformation of Syrian hamster embryo cells treated at 1 and 10 ppm.	In vitro transformation.	Dunkel et al. 1981 and Pienta et al. 1977
Amitrole	Hamster cells	Cell cultures treated with 10, 50, and 100 ppm.	Transformation observed at all dose levels tested.	In vitro transformation.	Inoue et al. 1981
Amitrole, Technical, 98% pure	Hamster cells	BHK21, C113/HRC1 line, treated with 0.2, 2.0, 200 and 2,000 ppm in presence or absence of S-9	No evidence of cell transformation.	—	Daniel and Dehnel 1981
Amitrole, Technical, 98% pure	Hamster cells	BHK21 C113 cells treated with 0.025, 0.25, 2.5, and 250 ppm.	Positive results for cell transformation.	—	Styles 1981

[a]Data not available or not applicable.

Appendix E

Summary of Amitrole Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole (50% pure)	Salmonella typhimurium	Tested with 8 unspecified strains of S. typhimurium at unspecified dose levels	Nonmutagenic	--[a]	Andersen et al. 1972
Amitrole	S. typhimurium	Strains TA98, TA100, TA1535, and TA1537, treated at unspecified dose levels in the presence and absence of S-9	Nonmutagenic	Summary information only.	McCann et al. 1975
Aminotriazole	S. typhimurium	Tested with strains TA1535, TA1536, TA1537, TA1538 at unspecified dose levels in the presence and absence of S-9	Nonmutagenic	Summary information only. No data presented.	Torracca et al. 1976
Amitrole, Technical	S. typhimurium	Tested with several trp$^-$mutants. Doses and conditions unspecified	Nonmutagenic	Summary information only. No data presented.	Sorsa and Gripenberg 1976
Amitrole Technical	S. typhimurium	Tested with strains TA1535, TA1536, TA1537 and TA1538 at unspecified dose levels	Nonmutagenic	Summary information only. No data presented.	Shirasu et al. 1976
Amitrole	S. typhimurium	Strains TA98, TA100 TA1535, TA1536, TA1537 and TA1538 treated with 250 µg/plate in the presence of S-9 fraction	Nonmutagenic	--	Simmon 1979
Amitrole	S. typhimurium	Strains TA1535 and TA1538 treated at dose levels of 25, 100, and 250 µg/plate, in the presence and absence of S-9	Nonmutagenic	--	Rosenkranz and Poirier 1979

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical	S. typhimurium	Tested with strains TA98, TA100, TA1535, TA1537 and TA1538 at half-log concentrations from 0.3 to 333.3 μg/plate, in the presence and absence of S-9	Nonmutagenic	Compound was tested in three laboratories under coded conditions by "state of art" techniques.	Dunkel 1979
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100, TA1535, TA1538 and TA92 treated with 0.2, 2.0, 20, 200, and 2,000 μg/plate, with preincubation in the presence and absence of rat liver S-9 fraction	Nonmutagenic	--	Brooks and Dean 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100 TA1535, TA1537, and TA1538 treated with up to a maximum dose level of 1 mg/plate in the presence and absence of rat liver S-9 fraction	Nonmutagenic	Summary information only.	Baker and Bonin 1981
Amitrole Technical, 98% pure	S. typhimurium	Strains TA98, TA100, and TA1537 treated at dose levels of 100, 200, 500, and 2,000 μg/plate, in the presence and absence of rat liver S-9 fraction	Nonmutagenic	Summary information only.	MacDonald 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98 and TA100 treated with unspecified dose levels in the presence and absence of S-9 fraction	Nonmutagenic	Summary information only.	Ichinotsubo et al. 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100 TA1537 treated with unspecified dose levels, in the presence and absence of S-9	Nonmutagenic	Summary information only.	Nagao and Takahashi 1981

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100, and TA1535 treated at dose levels of 10, 100, 1,000 and 10,000 µg/plate, in the presence and absence of S-9	Nonmutagenic	Summary information only.	Richold and Jones 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100, and TA1535 treated at dose levels of 0.1, 1.0, 10, 100, 500, and 2,000 µg/plate, in the presence and absence of S-9	Nonumutagenic	Summary information only.	Rowland and Severn 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100, TA1535, and TA1537 treated with unspecified doses in the presence and absence of S-9	Nonmutagenic	Summary information only.	Simmon and Shepherd 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA100, TA1535, TA1537 and TA1538 treated at dose levels of 4, 20, 100, 500 and 2,500 µg/plate	Nonmutagenic	Summary information only.	Trueman 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98 and TA100 treated at 0.5, 1.0, 5.0, 10, 50, and 100 µg/plate, in the presence and absence of S-9	Mutagenic	Summary information only.	Venitt and Crofton-Sleigh 1981
Amitrole, Technical 98% pure	S. typhimurium	Strain TM677, treated with dose levels up to 100 ppm, than tested for resistance to 8-azaguanine.	Nonmutagenic; no evidence of increased resistance to 8-azaguanine.	--	Skopek et al. 1981

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98, TA1535, and TA1537 tested in a microtiter fluctuation test with dose levels from 1 to 1,000 ppm in the presence and absence of S-9	Nonmutagenic	--	Gatehouse 1981
Amitrole, Technical, 98% pure	S. typhimurium	Strains TA98 and TA100 tested with doses of 10, 100, 200, and 500 ppm in the presence of rat hepatocytes	Questionable results ($p < 0.025$) noted at 500 ppm.	--	Hubbard et al. 1981
Amitrole, Technical, 95% pure	S. typhimurium	Tested with strains TA1535, TA1536, TA1537, and TA1538 at a level of 2,000 µg/plate in the presence and absence of S-9	Nonmutagenic	--	Carere et al. 1977 and Carere and Morpugo 1981
Amitrole, Technical	S. typhimurium	Tested in a host-mediated assay employing strain TA1950. Mice treated with amitrole alone or in combination with equimolar nitrite (1,450, 2,175, and 2,900 µmoles/kg)	Nonmutagenic	Weakly mutagenic in presence of nitrite indicating that the compound can be nitrosated to a mutagenically active form.	Braun et al. 1977
Amitrole, Technical	S. typhimurium	Tested in a host-mediated assay employing strains TA1530 and TA1538 in mice injected with 12,125 and 250 mg/kg	Mutagenic; increased frequency of mutation observed at 2 lowest doses with TA1530 and at middle dose with TA1538.	Not mutagenic at highest dose tested.	Simmon et al. 1979

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical	Escherichia coli	Tested with strains W3110 thy polA⁻ defective in DNA polymerase I and strain polA⁺	Nonmutagenic; no evidence of preferential inhibition	--	Bamford et al. 1976
Amitrole, Technical	E. coli	Tested with polA⁻ strain (defective of DNA polymerase). Doses and conditions unspecified	Nonmutagenic	Summary information only. No data presented.	Sorsa and Gripenberg 1976
Amitrole, Technical	E. coli	Tested with normal (polA⁺)and DNA polymorase I - deficient (polA⁻) strains at 250 ppm in the presence and absence of S-9	Nonmutagenic	--	Rosenkranz and Poirier 1979
Amitrole, Technical, 98% pure	E. coli	Strain WP2uvrA(p) (pKM101), treated at 10, 20, 50, 100, 200, and 500 μg/plate	Mutagenic	--	Venitt and Crofton-Sleigh 1981
Amitrole, Technical, 98% pure	E. coli	Strain WP2uvrA(trp⁻) WP2uvrA/pKM101(trp⁻). Doses unspecified	Nonmutagenic	Summary information only.	Matsushima et al. 1981
Amitrole, Technical, 98% pure	E. coli	Strains WP2, WP67 (uvrA PolA), and CM871 (uvrA lexA recA)tested with compound at up to 50 mg/mℓ	Nonmutagenic; no significant preferential killing observed	--	Tweats 1981
Amitrole Technical, 98% pure	E. coli	Strains W3110 (polA⁺) and P3478 (polA⁻) tested in a liquid suspension assay for preferential inhibition with 50 μℓ of chemical	Nonmutagenic	--	Rosenkranz et al. 1981

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical, 98% pure	E. coli	Strains WP2 (repair proficient) and WP67 uvrA PolA and CM871 uvrA recA lexA (repair deficient), tested with concentrations up to 2,500 ppm	Nonmutagenic, no evidence of preferential killing of repair deficient strains.	--	Green 1981
Amitrole, Technical, 98% pure	E. coli	Strain WP2 uvrA, tested in a microtiter fluctuation test with dose levels from 1 to 1,000 ppm in the presence and absence of S-9	Nonmutagenic	--	Gatehouse 1981
Amitrole, Technical, 98% pure	E. coli	A lysogenic strain of E. coli carrying prophage lambda was treated with amitrole at levels of 1, 2, 4, and 10 mg/mℓ in the presence and absence of S-9 and assayed for phage induction	Nonmutagenic; no evidence of induction of lambda phage was observed.	--	Thomson 1981
Amitrole, Technical	E. coli	Tested with strains B/R try WP2 and WP2 try hcr at unspecified dose levels	Nonmutagenic	Summary information only. No data presented.	Shirasu et al. 1976
Amitrole, Technical	Saccharomyces cerevisiae	Tested in a host-mediated assay employing strain D3 in mice treated orally with 5,000 mg/kg	Nonmutagenic	--	Simmon et al. 1979
Amitrole, Technical, 98% pure	S. cerevisiae	Strain D7 tested with dose level of 12.5 mg/mℓ in a mitotic gene conversion assay	Nonmutagenic	--	Zimmerman and Scheel 1981

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical, 98% pure	S. cerevisiae	Strain JD1, tested against a range of unspecified doses, in the presence and absence of rat liver S-9	Mutagenic; a significant increase in gene conversion above the controlled level was reported at a minimum dose of 300 ppm.	--	Sharp and Parry 1981[b]
Amitrole, Technical, 98% pure	S. cerevisiae	Strain D4, tested at dose levels of 0.33, 1.0, 20, 33.33, 100 and 333.33 μg/plate	Nonmutagenic	--	Jagannath et al. 1981
Amitrole, Technical, 98% pure	S. cerevisiae	Strains D6, tested at concentration of 50 ppm in the presence of S-9	Mutagenic; mitotic aneuploidy	--	Sharp and Parry 1981[b]
Amitrole, Technical, 98% pure	S. cerevisiae	Strain T1 and T2 tested at 1,000 ppm in presence of S-9, and at 100, and 1,000 g/mℓ in the absence of S-9	Nonmutagenic; no evidence of increased mitotic crossing over or preferential inhibition of a repair deficient strain.	--	Kassinova et al. 1981
Amitrole, Technical, 98% pure	S. cerevisiae	Strain XV185-14C tested with dose levels of 88.9 and 889 ppm	Nonmutagenic; no significant increases in His^+, Thr^+, and Arg^+ revertants.	--	Mehta and Borstel 1981
Amitrole, Technical, 98% pure	S. cerevisiae	Repair deficient strain rad 3, rad 18, rad 52, trp 2; and wild type (w+) strain 197/2d. Tested at dose levels of 100, 300, 500, and 750 ppm in the presence and absence of S-9	Mutagenic; inhibition of DNA repair observed at all doses tested.	--	Sharp and Parry 1981[a]

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical	Bacillus Subtilis	Tested with H17 Rec$^+$ and M45 Rec$^-$ strains at unspecified dose levels	Nonmutagenic	Summary information only. No data presented.	Shirasu et al. 1976
Amitrole, Technical, 98% pure	Bacillus subtilis	Spores of strains H17rec$^+$ and M45 rec$^-$ were treated with amitrole (1 mg/disc), in the presence and absence of rat, yellow-tail fish and clam S-9	Mutagenic; differential inhibition of repair deficient M45 rec$^-$ strain was observed when compound was tested in the presence of S-9 prepared from yellowtail fish or clam.	Rat S-9 did not activate amitrole in this system.	Kada 1981
Amitrole, Technical, 95% pure	Streptomyces coelicolor	Tested at 1,000 µg/plate for resistance to low levels of streptomycin	Weakly mutagenic	--	Carere et al. 1976, 1978 and Carere and Morpugo 1981
Aminotriazole	S. coelicolor	Tested for resistance to streptomycin at unspecified dose levels of amitrole	Mutagenic	Summary information only. No data presented.	Torracco et al. 1976
Amitrole, Technical, pure standard	Aspergillus nidulans	Tested with strains 35 and P at 2,000 µg per plate	Nonmutagenic for point mutations.	Mitotic segregation (crossing-over and non-disjunction) was observed.	Bignami et al. 1977
Amitrole (50% pure)	Bacteriophage	Tested with E. coli bacteriophage T_4 and and two mutants of T_4 at 50-1,000 µg/plate	Nonmutagenic	--	Andersen et al. 1972
Amitrole Technical	E. coli phage	Tested with two strains of E. coliphage, λ and λcIts 857, at concentrations up to 40,000 and 15,000 ppm with and without prior metabolic activation	Induced prophage λcIts 857 but not λ, and only in presence of metabolic activation.	A novel system tested with a limited number of chemicals.	Ho and Ho 1979

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole, Technical	EUE cells	Tested for induction of unscheduled DNA synthesis at unspecidose levels in cells *in vitro*	Positive for unscheduled DNA synthesis.	Only summary statement given. No data presented.	Benigni and Dogliotti 1980a, 1980b
Amitrole, Technical, 98% pure	HeLa cells	Tested for induction of unscheduled DNA repair synthesis. Cells treated with 0.1 to 100 ppm test material in the presence and absence of rat liver S-9 fraction	Positive in presence of S-9 fraction.	--	Martin and McDermid 1981
Amitrole, Technical 98% pure	Chinese hamster cells	CHO cells treated 1 hr in the presence and absence of S-9, or treated for 24 hours. Dose levels were 0.01, 0.1, 1.0, 10, and 100 ppm	Nonmutagenic; no evidence of sister chromatid exchanges.	--	Perry and Thomson 1981
Amitrole, Technical	Chinese hamster cells	CHO cells treated with 10^{-3} and 10^{-4} molar concentrations	Negative for sister chromatid exchanges.	--	Athanasiou and Kyrtopoulus 1981
Amitrole, 96.3% pure	*Drosophila melanogaster*	Fed at (10 ppm) 0.001% in diet for nondisjunction test and sex-linked recessive lethal test	Nonmutagenic	--	Laamanen et al. 1976
Amitrole, Technical	*D. melanogaster*	Tested with Oregon-R strain; 0.001% amitrole in diet	Nonmutagenic; no evidence of sex-linked recessive lethals in males and sex chromosome nondisjunction in females.	--	Sorsa and Gripenberg 1976
Amitrole, Technical, 98% pure	*D. melanogaster*	Compound tested at 0.2% concentration in the diet	Nonmutagenic in the sex-linked recessive lethal test.	--	Vogel et al. 1981

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Amitrole	Human lymphocytes	Tested at concentrations of 0.0001% to 1.0% (0.1 to 1,000 ppm)	Nonmutagenic; no clastogenic effect, i.e., no evidence of damage to chromosomes.	--	Meretoja et al. 1976
Amitrole, Technical	Human lymphocytes	Cells treated in culture with concentrations exceeding 0.2% w/v	Nonmutagenic; no evidence of increase in aneuploidy or structural changes in chromosomes.	--	Sorsa and Gripenberg 1976
Amitrole, Technical, 98% pure	Mouse	CD-1 mouse bone marrow cells from animals treated with 125, 250, and 500 mg tests material per kg body weight	Nonclastogenic; no evidence of chromosome aberrations when bone marrow cells examined for micronucleated polychromatic erythrocytes.	--	Tsuchimoto and Matter 1981
Amitrole, Technical, 98% pure	Mouse	Strain B6C3F, bone marrow cells examined after treatment of mice at dose levels of 1,800 to 2,800 μg test compound	Nonclastogenic	Initial weak response after treatment with 2,800 μg compound could not be confirmed when retested at 1,800, 2,400 and 3,000 μg.	Salamone et al. 1981
Amitrole, Technical, 98% pure	Mouse cells	Strain C3H2K cells treated with compound at 0.1 to 10 μg/dish, then infected with murine leukemia virus (MLV)	Negative response. No evidence of enhanced infection of C3H2K cells.	--	Yoshikura and Matsusihima 1981

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Amitrole

Formulation	Organism	Dose	Comments	References
Amitrole (^{14}C) (3-Amino-1,2,4-triazole-[5-C^{14}])	Rat	50 mg/kg body weight, single dose	Most of the administered dose was excreted in the urine within 24 hours. Six percent of the dose was metabolized to other compounds within 3 days.	Grunow et al. 1975
Amitrole (^{14}C) (3-Amino-1,2,4-triazole-[5-C^{14}])	Rat	1 to 200 mg/rat via stomach tube	From 70.0 to 95.5% of the total radioactivity administered was found in urine within 24 hours. Feces contained a small but variable amount of activity. Tissues absorbed material rapidly, reaching a maximum within 1 hour, remaining constant for 2 to 3 hours, and then decreasing with a halflife of 2 to 3 hours.	Fang et al. 1964, 1966
Amitrole	Human (adult female)	20 mg/kg body weight	100 mg amitrole per 100 mℓ urine was recovered several hours after ingestion, representing 50% of the estimated intake.	Geldmacher-v. Mallinckrodt and Schmidt 1970
Amitrole	Rats	Animals injected with amitrole for 8 days	Unaltered amitrole appeared in the urine within 24 hours of first injection, and amitrole and metabolite excretion ceased within 24 hours of final injection.	Franco and Municio 1975

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Herbicide Background Statement: Atrazine

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Atrazine Summary

Major Trade Names: AAtrex®, Co-op, Co-op Atra-pril

Chemical Name: 2-chloro-4-ethylamino-6-isopropylamino-s-triazine

Major Forestry Applications: Applied as a selective herbicide for conifer release, site preparation, grass and noxious weed control, and management of wildlife habitat and rights-of-way.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Atrazine is generally nontoxic or only slightly toxic to a variety of animal species, although it is toxic to some aquatic organisms at concentrations below 10 ppm. Atrazine has very low toxicity to birds and fish. The acute oral toxicity is approximately 3,000 mg/kg in rats and 1,750 mg/kg in mice. No observable adverse effects were detected in sheep and calves fed a diet containing 760 ppm atrazine or cattle fed daily doses of 100 ppm for three weeks. Rats fed triazine at dietary levels of 100 and 500 ppm for six months exhibited growth retardation, which was attributed in part to a reduction of food intake. Atrazine was nontoxic to rats fed diets containing atrazine at levels up to 100 ppm for two years. Application of atrazine (80% wettable powder) to the skin of rats showed an LD_{50} of 9,300 $\pm$ 990 mg/kg, and repeated applications of 2,800 mg/kg produced redness of the skin but no systemic effects. Rats exposed to aerosol dust containing 1.6 ppm active ingredient produced no evidence of toxicity.

Feeding atrazine at levels up to 1,000 ppm to pregnant rats throughout gestation had no effect on the number of offspring or their weight at weaning. Subcutaneous injections of 800 mg/kg or higher were embryotoxic. Atrazine was generally nonmutagenic in the Ames Salmonella/microsome test and a number of microbial mutagenicity tests. In a few instances, positive results were obtained when atrazine was tested in the presence of extracts prepared from plants such as potato or Nicotiana. The relevance of these results for potential hazards to animals is unclear. Equivocal results were obtained in mutagenicity tests employing the fruit fly. Atrazine was noncarcinogenic when fed to mice at 82 ppm in the diet for 18 months. Gross and microscopic examination revealed no significant increase and distribution of tumors when compared with controls.

Environmental Fate

Atrazine is absorbed primarily by plant roots, readily translocated to all above-ground parts of the plant, and accumulates in the margins of leaves. It is readily metabolized by plants by a variety of pathways. It has a low tendency to bioaccumulate in animals and is rapidly metabolized to nontoxic metabolites that are readily excreted via the kidneys.

Atrazine is moderately persistent in soil, from which it is lost primarily by means of chemical and microbial degradation. It is moderately mobile and adsorbs readily to soil particles, particularly in soils with high organic matter content. Atrazine is only slightly soluble in water and its low solubility, coupled with a tendency to adsorb to soil particles, results in only relatively small amounts of atrazine in water. It degrades slowly in water and therefore is lost by dilution, transport, and metabolism by organisms.

Exposure and Hazard

The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels anticipated for Forest Service applications. There should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from atrazine use, if proper care is taken during application.

1.0 General Information

Atrazine has, since discovery of its herbicidal properties in the early 1950's, been widely used for selective applications in crop, range, and forest lands and for nonselective vegetation control applications in noncrop lands. In the United States, atrazine is also known by the trade names* AAtrex®, Co-op, and Co-op Atra-pril. It is formulated with other herbicides under other trade names. Atrazine is absorbed through both roots and foliage and is translocated through the plant to leaves and to areas of cell proliferation where it acts primarily as a photosynthetic inhibitor (Ghassemi et al. 1981 and Weed Science Society of America 1983).

1.1 Normal Use Patterns

Atrazine has been among the most widely used herbicides in the United States since its introduction in 1958. Atrazine is used on crops such as corn, sorghum, sugar cane, and pineapple and on conifers for pre- or post-transplant applications. Atrazine is used in forestry applications for conifer release, site preparation, grass and noxious weed control, and in management of wildlife habitat and rights-of-way (Gross 1983). Application rates ranged from 0.5 to 5.0 lbs active ingredient (a.i.) per acre for 41 USDA Forest Service projects in which atrazine was used in 1982. Atrazine is usually applied at a rate of 2 to 4 lbs a.i./acre (2.24-4.48 kg/ha) for selective weed control applications, and at higher rates for nonselective weed control applications. For some weed control uses, particularly in rangeland applications and applications against common annual broadleaf weeds, lower rates can be effectively utilized (Weed Science Society of America 1983). Statistics on the use of atrazine by the USDA Forest Service are presented in Table 1-1.

Atrazine is manufactured and marketed in the United States by the Ciba-Geigy Chemical Corporation under the trade name AAtrex® and is available as wettable powders, water dispersible granules, and liquid suspension (Esser et al. 1975 and Weed Science Society of America 1983). Abroad, atrazine is manufactured and/or marketed under a variety of trade names by several companies including Ciba-Geigy Chemical Corporation. Atrazine is variously formulated with other herbicides, including alachlor, propachlor, sodium chlorate and sodium metaborate, prometon, and metolachlor for a variety of uses (Ghassemi et al. 1981 and Weed Science Society of America 1983).

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

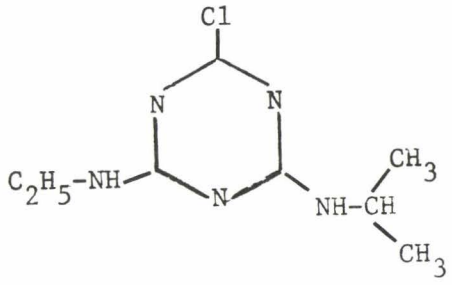
USDA Forest Service Atrazine Use

Purpose	Active Ingredient Per Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
		1981			
Aerial					
Conifer release	4.0	4.0	1,152	Liquid	Helicopter
Site preparation	3.4-5.6	5.1	2,649	Liquid	Helicopter
Ground					
Conifer release	2.0-4.0	2.6	380	Liquid	Broadcast
Grass control	1.7	1.7	103	Liquid	Broadcast
Noxious weeds	1.2	1.2	95	Liquid	Broadcast
Right-of-way	2.1	2.1	205	Liquid	Broadcast
Site preparation	1.0-8.0	1.3	1,370	Liquid	Spot Broadcast
		1982			
Aerial (7 projects)					
Timber	3.0-4.0	3.8	7,852	Liquid	Aerial
Ground (34 projects)					
Nursery, Timber, Research, Wildlife, Noxious Weeds, Engineering	0.5-5.0	3.3	4,350	Liquid	Ground foliar

Source: Gross 1983.

1.2 Physical and Chemical Properties

The effective ingredient of atrazine and its formulations is 2-chloro-4-ethylamino-6-isopropylamino-s-triazine, with the following structural formula (Esser et al. 1975):



Atrazine has a molecular formula of $C_8H_{14}ClN_5$ and a molecular weight of 215.7. In its pure form, atrazine is a white, crystalline solid with a melting point of 343.4 to 347°F (173 to 175°C). Atrazine is relatively insoluble in water, having a solubility of 33 ppm by weight at 80.6°F (27°C). It is far more soluble in organic solvents than in water, having solubilities of 183,000, 52,000, 28,000, 18,000, 12,000, and 360 ppm by weight in dimethyl sulfoxide, chloroform, ethylacetate, methanol, diethylether, and n-pentane, respectively, at 80.6°F (27°C) (Weed Science Society of America 1983).

Atrazine is degraded by both biological and nonbiological mechanisms. Jordan et al. (1970) reviewed the processes by which atrazine and other s-triazines are degraded, including photodecomposition, volatilization, hydroxylation, and dealkylation. Laboratory studies have shown that exposure of atrazine to both ultraviolet and infrared light results in loss of herbicidal activity due to decomposition. However, data from field studies and from soil-applied atrazine in the laboratory indicate that other environmental factors, such as leaching below light penetration zones in soil, affect the actual rate of atrazine photodegradation under natural conditions. Atrazine has a relatively low vapor pressure, ranging from 5.7×10^{-8} mm Hg at 50°F (10°C) to 2.3×10^{-5} mm Hg at 122°F (50°C) (data furnished by Geigy Chemical Corporation in Jordan et al. 1970). However, although volatilization of atrazine does not appear to be a major factor for its dissipation from soil, it does appear that volatilization is a major factor for atrazine dissipation from foliage (Burt 1974). Atrazine is readily hydrolyzed by a nonbiological reaction to form a nonphytotoxic metabolite, hydroxyatrazine. Finally, there is evidence that dealkylation by nonbiological free radical reactions in soil environments act to degrade atrazine.

The physical and chemical properties of atrazine have had a profound influence on the nature of atrazine formulations.

Specifically, liquid formulations with high concentrations of atrazine in water are precluded by the low solubility of atrazine. Consequently, wettable powders which also permit ease of transport, measurement, and application have been developed. These powders, in commercial form, contain as much as 80 percent atrazine or mixtures of atrazine with other herbicides. In addition to atrazine, such powders contain wetting agents, dispersing agents, and inert chalk or clay to make up the bulk of the formulation. Granular formulations of atrazine permit application without the added bulk or weight of water, and are particularly useful in large-scale ground and aerial applications (Esser 1975); however, none are registered for use in forestry applications.

2.0 Toxicity of Atrazine

The toxicological properties of atrazine, summarized in Table 2-1, show that this herbicide is generally nontoxic or only slightly toxic to a variety of animal species, although a few studies found toxic effects, particularly in aquatic organisms. Atrazine is relatively toxic to a wide range of nontarget plant species in both forest and crop applications. Studies with rodents indicate that atrazine is nonteratogenic; has little or no effect on fertility, reproduction, or development of offspring; is nonmutagenic; and appears to present little or no carcinogenic risk. In fish and amphibians, however, teratogenic effects have been shown at doses of 5 to 25 ppm atrazine.

2.1 Invertebrates and Microorganisms

Atrazine toxicity has been determined in a wide variety of invertebrate species and microorganisms from terrestrial, marine, and freshwater ecosystems. Major findings of these studies are presented in Appendix A and indicate a wide variety of toxic responses ranging from a 48-hour LC_{50} of 0.72 ppm in midge larvae (Macek et al. 1976) to a chronic dietary tolerance of 1,000 ppm in the cockroach (Riviere 1976). In general, the data indicate that atrazine is only slightly toxic to most terrestrial and intertidal marine organisms, in which species toxic effects are generally observed at levels far greater than 10 ppm. In aquatic invertebrates, however, toxic effects generally appear at concentrations below 10 ppm, and for some species below 1 ppm. In aquatic organisms, effects at concentrations above 10 ppm are considered indicative of highly toxic compounds, effects at concentrations between 1 and 10 ppm are considered indicative of toxic compounds, and effects at concentrations below 1 ppm are indicative of highly toxic compounds (Clarke et al. 1970).

Two cautions should be borne in mind when considering atrazine toxicity data. First, toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of atrazine in a natural environment. For example, in the laboratory, aquatic organisms were kept for as long as 120 days (in some cases continuously for several generations) with continuous exposure to atrazine. Microorganisms were grown in a culture medium containing atrazine, often at concentrations several orders of magnitude greater than those normally found in the environment following application. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to atrazine or other man-made chemicals is usually a transient phenomenon whose duration depends on the rate of streamflow and on the rate of chemical decomposition of the chemical. Additionally, in natural

Table 2-1

Summary of Toxicological Properties of Atrazine[a]

1. Acute Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Atrazine)	Ingestion of a single dose	LD_{50} = 1,400 to 3,000 mg/kg
Rat (AAtrex®)	Ingestion of a single dose	LD_{50} = 3,080 mg/kg
Rat (Atrazine 80W)	Ingestion of a single dose	LD_{50} = 5,100 ± 400 mg/kg
Mouse (Atrazine)	Ingestion of a single dose	LD_{50} = 1,750 mg/kg
Fish, various species of adults (various formulations)	96 hours	LC_{50} > 1.0 ppm

2. Acute Intraperitoneal Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Atrazine)	Single intraperitoneal injection	LD_{50} = 125 mg/kg

3. Acute Dermal Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (AAtrex® (80W))	Application to skin	LD_{50} = 9,300 ± 990 mg/kg

4. Skin Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rat (Atrazine)	Unspecified	2,800 mg/kg produced erythema but no systemic effects

Table 2-1 (Continued)

5. Inhalation Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Atrazine)	Animals exposed for unspecified time to aerosol dust containing 1.6 ppm active ingredient	No evidence of toxicity.

6. Primary Eye Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rat	Dose and conditions for exposure unspecified	"Irritating"

7. Subchronic Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Atrazine)	Fed at dietary levels of 100 and 500 ppm for 6 months	Retardation of growth due in part to decreased food intake.
Cattle (Atrazine (80W))	Fed at dietary level of 100 ppm for 21 days or 30 ppm for 28 days	No observable effects.
Sheep (Atrazine)	Fed on hay containing approximately 500 and 760 ppm for 4 weeks	No significant adverse effects.
Birds (Atrazine)	Fed up to 5,000 ppm in feed for 5 days.	No mortality to 5,000 ppm for bobwhite, Japanese quail, or ring-necked pheasant, and mallards. Thirty percent mortality at 2,500 ppm.

8. Chronic Toxicity

Organisms (Formulation)	Exposure	Adverse Effects
Rat	Fed at dietary level of 100 ppm for 2 years	No gross or microscopic effects observed.

Table 2-1 (Continued)

9. Reproduction and Teratogenicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Atrazine)	Fed at dietary levels of 50 to 1,000 ppm from day 1 throughout gestation.	No effects on number of pups per litter or weight at weaning.
Rat (Atrazine)	Subcutaneous injections at levels of 50 to 2,000 mg/kg on days 3, 6 and 9 of gestation.	Levels of 800 mg/kg or higher embryotoxic, with resorptions occurring in most of the litters.

10. Carcinogenicity

Organism (Formulation)	Exposure	Adverse Effects
Mouse (Atrazine)	21.5 mg/kg by stomach tube from days 7 through 28 followed by 82 ppm in diet for 18 months	No gross or microscopic induction of tumors related to treatment.
Mouse (Fogard S®); 25% atrazine, 37.5% simazine)	13 subcutaneous injections for total of 0.0065 mg a.i.; 13 intraperitoneal injections for total of 0.0065 mg a.i.	Apparent increase in incidence of lymphomas.

11. Mutagenicity

Organism (Formulation)	Adverse Effects
Salmonella typhimurium (Atrazine)	Except for one report, atrazine was nonmutagenic when tested at doses up to 5 mg/plate in the presence or absence of mammalian metabolic activation.
Saccharomyces cerevisiae (Atrazine)	Atrazine alone was generally nonmutagenic when tested in the presence of mammalian or plant metabolic activation systems. An increase in frequency of mitotic gene conversion was observed when cultures of the yeast were treated with extracts of maize plants treated with atrazine.
Escherichia coli (Atrazine)	Equivocal results. Mutagenic in a forward mutation host-mediated test in one laboratory but negative in another laboratory.

Table 2-1 (Concluded)

11. Mutagenicity (Concluded)

Organism (Formulation)	Adverse Effects
Aspergillus nidulans (Atrazine)	Generally nonmutagenic for atrazine itself. Metabolite from treated Nicotiana plants give positive results for 8-azaguanine resistance and mitotic crossing over.
Schizosaccharomyces pombe (Atrazine)	Mutagenic when tested in the presence of potato microsomes or animal S-9 fraction for metabolic activation.
Streptomyces coelicolor (Atrazine)	Mutagenic when tested in the presence of potato microsomes.
Bacteriophage T_4 and rII mutants of bacteriophage T_4	Nonmutagenic
Drosophila melanogaster	Increase in rate of dominant lethals, X-linked recessive lethals.
V79 hamster cells	Mutagenic for resistance to 6-thioguanine when tested in the presence of potato microsomes.
Chinese hamster cells	No cytogenetic damage when tested with or without metabolic activation. No increase in sister chromatid exchanges.
EUE human cells	Positive for unscheduled DNA synthesis when tested in the presence of potato microsomes.
Mouse bone marrow cells	Increase in chromosome aberrations.
Mouse	Induction of dominant lethal mutations when mice were dosed orally with 1,500 and 2,000 mg atrazine/kg body weight.
Barley seeds or seedlings	Induced chromosomal aberrations in root top cells.
Maize seedling roots	Increased frequency of sister chromated exchanges in root cells.
Maize plants	Increased frequency of reversion at the waxy locus in pollen grains from plants grown in the presence of atrazine.

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

systems, interaction with other man-made and naturally occurring chemicals and changes in environmental factors may affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions, in turn, potentially alter the tolerance levels of organisms to the herbicides. Secondly, atrazine has a relatively low solubility in water (33 ppm at 80°F [Weed Science Society of America 1983]). Any toxicities in excess of 30 to 40 ppm atrazine in water are a measure of both dissolved and undissolved atrazine or of the solvent, such as methanol, used to dissolve the atrazine prior to mixing aqueous solutions for toxicity studies. For the most part, in actual field situations, aquatic organisms should rarely, if ever, be exposed for long periods of time to atrazine concentrations much in excess of atrazine solubility.

Insect studies with atrazine, summarized in Appendix A, report lethal effects ranging from a 48-hour LC_{50} of 0.72 ppm in midge larvae (Macek et al. 1976) to an 18-hour stressed (0.5 percent methanol in a 25 ml test system) LC_{50} 200 ppm in the mosquito (Bowman et al. 1981). A feeding study with the cockroach determined that 1,000 ppm in the diet through two generations had negligible effects on growth and reproduction (Riviere 1976). Nonlethal effects reported by Macek et al. (1976) included reduced hatching success, increased larval mortality, retarded development, and a reduced rate of both pupation and emergence in midge larvae exposed through two generations to 0.23 ppm or more of atrazine. Atrazine is reported not to have insecticidal activity (Weed Science Society of America 1983). Specific data on beneficial insects, such as honeybees, are not available.

Numerous studies have shown a wide range of toxicity values for atrazine in freshwater, marine, and intertidal crustaceans ranging from an 18-hour LC_{50} of 0.6 ppm for Daphnia stressed with 0.4 percent methanol in a 25 ml test system (Bowman et al. 1981) to no observable effects in fiddler crabs exposed for 8 weeks in early winter to 100 ml of a 1,000 ppm atrazine suspension (Plumley et al. 1980). In general, studies with aquatic crustacea (scuds, Daphnia, shrimp, and gammarid amphipods) indicated LC_{50}'s between 1 and 10 ppm. Studies with fiddler crabs and shore crabs (intertidal crustaceans) indicated no lethal effects at concentrations as high as 100 ppm atrazine. A seasonal study with the fiddler crab (Plumley et al. 1980) indicated that adverse effects noted at 100 ppm atrazine in a study in August diminished with new crabs in subsequent tests so that by November, no adverse effects were noted at 1,000 ppm atrazine.

Studies with molluscs indicated variable responses to atrazine. In freshwater pond enclosures with atrazine doses of 0.5 and 2.0 ppm in the water, clam populations were reduced to 1/8 of their original number while snail populations increased by a factor of 4 (Walker 1962 in Pimentel 1971). This increase in snail populations may have

resulted from reduced competition due to decreased numbers of individuals in populations of potential competitors. In marine molluscs, eastern oysters suffered no noticeable effect on shell growth after 96 hours exposure to 1 ppm atrazine (Butler 1965) and the cockle was determined to have an LC_{50} in excess of 100 ppm (Portmann and Wilson 1971 in Ghassemi et al. 1981).

In studies with microorganisms, including soil and aquatic species, atrazine was generally found to be more toxic to aquatic phytoplankton (such as algae) than to soil microorganisms (bacteria, fungi, actinomycetes and algae). In a study with soil amoeba, Pons and Pussard (1980) reported that only 2 of 22 strains were retarded in their growth when atrazine was incorporated into culture media at rates of up to 70 ppm. Prescott and Olson (1972), however, found reduced growth in cultures of the soil amoeba Acanthamoeba castellani at 0.1 ppm atrazine and arrested growth at 1 and 10 ppm. In terms of soil microorganisms (primarily bacteria, fungi, and actimomycetes) Mukhopadhyay (1980) found reduced growth and decreased production CO_2 (CO_2 production is a sign of microorganism metabolic activity) 5 days after application of 0.89 lb a.i. atrazine per acre. He found a return to growth and activity equivalent to that of controls after 15 days. Richardson (1970) reported reduced growth in some species of soil fungi exposed to 4.4 to 140 ppm atrazine incorporated into a culture medium. Stojanovic et al. (1972) also found growth inhibition and reduced CO_2 production when microogranisms were treated with atrazine, but they applied the equivalent of 5 tons a.i./acre. Other studies with microorganisms demonstrated the ability of such organisms to metabolize atrazine (Schocken et al. 1982 and Giardina et al. 1980). In terms of soil algae, Loeppky and Tweedy (1969) found conflicting responses with two species each of the genera Chlamydomonas and Chlorella, with one species of each having a marked toxic response to atrazine and one species having only a slight response. Other authors (Karlander et al. 1983; Hutber et al. 1979; Butler et al. 1975a and 1975b) found atrazine to reduce growth of aquatic algae (including blue-green algae) at concentrations of 1 ppm or less.

2.2 Fish and Amphibians

The toxic effects of atrazine and several of its formulations on fish and amphibians are summarized in Appendix B. In most cases, adult fish LC_{50}'s were reported to be greater than 1.0 ppm and, in many instances, greater than 10 ppm. For adult fish, 96-hour LC_{50}'s for immersion ranged from 0.55 ppm in the harlequin fish (Alabaster 1969 in Pimentel 1971) (all other adult LC_{50}'s 4.5 ppm) to greater than 100 ppm in the Crucian carp (Bathe et al. 1976). For many species of fish, the LC_{50}'s of fry were lower than adults and ranged

from an LC_{50} of 0.22 ppm for the channel catfish (Birge et al. 1979 and 1983) to a report of no mortality at 11 ppm for Lake chubsucker fry (Hiltibran 1967). Clark et al. (1970) suggest that pesticides with median lethal concentrations of 10 ppm or more for aquatic species be considered "slightly toxic," between 1 and 10 ppm be considered "toxic," and less than 1 ppm be considered "highly toxic." Thus, atrazine should be considered toxic to highly toxic to fry of most species of fish, and slightly toxic to toxic for adults of most species of fish.

Atrazine toxicity studies with amphibians have included egg and early tadpole stages of frogs and toads (Birge et al. 1980 and 1983 and data cited in Hine et al. 1981) and a report of lethal thresholds* in developing larvae of leopard frogs (Hovey 1975 in Hine et al. 1981). These studies, summarized in Appendix B, indicate that LC_{50}'s from spawning through 96-hours post hatching (4-day old tadpoles) ranged from 0.41 ppm in the bullfrog to greater than 48 ppm in the American toad (both extremes from Birge et al. 1980 and 1983). With the exception of the low bullfrog toxicity cited, LC_{50}'s for frog and toad eggs and tadpoles were greater than 5 ppm, indicating that atrazine is toxic to slightly toxic to amphibian eggs and tadpoles.

Atrazine was found to have teratogenic effects, particularly manifested as spinal cord and other skeletal malformations, in fish fry and in frog tadpoles that had hatched from eggs exposed to lethal concentrations (Birge et al. 1979, 1980 and 1983). In channel catfish eggs exposed to 4.83 ppm atrazine, 69 percent of the eggs that hatched displayed teratogenic effects. Sixty-two percent of rainbow trout eggs exposed to 5.02 ppm atrazine hatched into teratogenic fry. For both species, these concentrations are well above the median lethal concentrations for eggs kept from spawning through 4-days post-hatch (0.22 and 0.87 ppm for channel catfish and rainbow trout, respectively). Teratogenic effects in 97 percent of the hatched tadpoles were observed when bullfrog eggs were kept in 24.4 ppm atrazine. For American toad eggs exposed to a maximum of 48.2 ppm atrazine, 17 percent of the eggs that hatched resulted in teratogenic effects. The median lethal concentrations for eggs of bullfrogs and American toads kept in atrazine from spawning through 4-days post hatching were 0.41 and >48 ppm, respectively.

2.3 Birds

Dietary studies and egg injection bioassays with atrazine are summarized in Appendix C and indicate a low toxicity to birds. Adverse affects ranged from reduced weight gain in chickens dosed at

*Lethal threshold is the concentration at which one more individual died in the exposed group than in the control group.

50 mg/kg for 10 days by capsule (Palmer and Radeleff 1969) to an LC_{50} of 19,650 ppm in feed in an 8-day dietary study with mallards reported by Ciba-Geigy Chemical Corporation (Weed Science Society of America 1983).

In general, dietary studies indicated a low toxicity of atrazine to bird species. Results of 8-day feeding studies (usually reported as 5 days ingestion of atrazine in diet followed by 3 days of observation) ranged from an LC_{50} of 700 to 800 ppm in bobwhite (Heath et al. 1972 in Ghassemi et al. 1981 and in U.S. Department of Energy 1983) to LC_{50}'s greater than 5,000 ppm for bobwhite, Japanese quail, ring-necked pheasant and mallard (Hill et al. 1975) and the LC_{50} of 19,650 ppm in mallards (Ciba-Geigy Chemical Corporation data reported in Weed Science Society of America 1983) cited above. In a 14 day ingestion study with female chickens, 100 ppm in feed for 7 days, followed by 7 days observation, indicated no adverse effects (Foster and Khan 1976). In longer-term studies, chickens treated with 10 daily doses of 50 mg/kg had 24 percent weight gain, compared to 45 percent gain in untreated controls and 2 percent loss in chickens fed 250 mg/kg (Palmer and Radeleff 1969). However, in pheasant hens fed 15 weekly doses of up to 400 mg atrazine, there were no adverse effects in terms of weight gain, number of eggs laid, or eggshell thickness (Melius 1975). The offspring of these hens were not adversely affected in terms of sensory-motor behavior (visual-cliff performance), survival, or weight gain. However, eggs of hens treated at 200 and 400 mg were lighter than controls, chicks of hens treated at 100 mg were more readily captured than controls or than chicks from more heavily dosed hens, and there was an increase in fertility and in hatchability of eggs in hens fed low doses of atrazine compared to both controls and to hens fed high doses.

In an egg injection study, Dunachie and Fletcher (1970) injected atrazine into chicken eggs to give concentrations of 10, 100, 200, 300, and 400 ppm in the egg. Reduced hatching (73 percent of control) occurred only for eggs with 400 ppm atrazine. No teratogenic effects, such as feather blanching, or malformation of beak, limbs, or spine, were observed in hatchlings from atrazine-treated eggs.

2.4 Mammals

The potential health hazards from exposure to atrazine have been evaluated in various species of mammals. Studies have been done mostly with mice, rats, and dogs. Few studies on sheep, cows and horses were reported. No studies were available on wildlife mammals.

Results of the various toxicity tests are described below and summarized in Appendix D and Table 2-1.

2.4.1 Acute Toxicity

Acute toxicity data have been reported for atrazine administered by oral, intraperitoneal, dermal, inhalation, and ocular routes.

2.4.1.1 Oral. Atrazine has low toxicity in rats and mice. In rats, the oral LD_{50} ranged from 1,400 to 3,000 mg/kg (Gzhegotskiy et al. 1977 and Bashmurin 1974 in Hayes 1982). The LD_{50} for atrazine formulated as AAtrex® was 3,080 mg/kg (Geigy Agricultural Chemicals 1971 in U.S. Department of Agriculture 1978) and for Atrazine 80W was 5,100 mg/kg (Geigy Agricultural Chemicals 1971b). In mice, the oral LD_{50} for atrazine and AAtrex® was 1,750 mg/kg (Bashmurin 1974 in Hayes 1982 and Geigy Agricultural Chemicals 1971a). Atrazine is much more toxic when given by routes other than oral. The LD_{50} of atrazine given to rats by the intraperitoneal route was reported to be 125 mg/kg (Gzegotskiy et al. 1977 in Hayes 1982).

2.4.1.2 Dermal. When applied to the skin of rats, the LD_{50} for AAtrex (80W) was reported to be approximately 9,000 mg/kg (Geigy® Agricultural Chemicals 1971b). In skin irritation tests, a dose of 2,800 mg/kg of the same compound produced erythema but no systemic effects (Gzhegotskiy et al. 1977 in Hayes 1982).

2.4.1.3 Inhalation. According to a summary statement alluding to an inhalation study, rats exposed to an aerosol dust containing 1.6 ppm active ingredient atrazine produced no evidence of toxicity (Geigy Agriculture Chemicals 1971a).

2.4.1.4 Eye Irritation. Atrazine was reported to be "irritating" to the eyes when tested in rats (Gzhegotskiy et al. 1974 in Hayes 1982). No details regarding dosages or test conditions were presented in the secondary literature source.

2.4.2 Subchronic Toxicity

2.4.2.1 Oral. Administration of daily dosages of 100 ppm of an 80 percent wettable powder formulation of atrazine to cows for 21 days, or feeding 30 ppm of this formulation in grain to cattle for four weeks, resulted in no observable effect (Geigy Agricultural Chemicals 1971a).

When rats were fed atrazine at dietary levels of 100 and 500 ppm for 6 months, growth retardation was observed. This retardation in growth was attributed in part to a reduction of food intake by the animals (Suschetet et al. 1974).

No significant adverse effects were observed when sheep were fed hay containing approximately 500 and 760 ppm for a total of 865 and 1,200 mg/kg body weight over a 4-week period (Johnson et al. 1972).

Calves similarly fed hay containing approximately the same content of atrazine showed no adverse effects after 4 weeks (Suschetet et al. 1974).

2.4.3 Chronic Toxicity

Atrazine fed to rats at dietary concentrations of 100 ppm for 2 years produced no gross or microscopic signs of toxicity (National Research Council 1971). In another report (Geigy Agricultural Chemicals 1971a), no ill effects were observed when rats, dogs, horses, and cattle were fed atrazine at 25 ppm over "extended periods."

2.4.4 Reproductive and Teratogenic Effects

No multigeneration reproductive studies on atrazine are available. Peters and Cook (1973) tested the effect of atrazine on reproduction in rats. In one experiment, pregnant rats were fed atrazine at dietary levels of 50 to 1,000 ppm from day 1 throughout gestation. No effect on reproduction, as reflected by the number of pups per litter or their weights at weaning, was observed. In another series of experiments, pregnant rats were injected subcutaneously with atrazine at levels of 50, 100, 200, 800, 1,000 and 2,000 mg/kg body weight on days 3, 6 and 9 of gestation. No effect on the number of pups per litter was observed at levels up to 200 mg/kg. Doses of 800 mg/kg or higher were embryotoxic. Resorptions of embryos occurred in most of the litters at these doses. Exposure on day 6 appeared to be critical for the induction of effects on organogenesis.

When pregnant mice were dosed with atrazine at 46.4 mg/kg from days 6 through 14 of gestation, no teratogenic effects were observed in the three strains of mice tested (Mrak 1969 in Hayes 1982).

In a study in farm animals, ewes were treated with atrazine at levels up to 30 mg/kg throughout pregnancy. Ewes treated with 15 mg/kg delivered normal lambs. The ewes were treated for an additional 30 days during which the lambs nursed without any apparent toxic effects. The dosage of 30 mg/kg was embryotoxic and fatal to pregnant as well as nonpregnant ewes (Binns and Johnson 1970).

2.4.5 Carcinogenicity

In a long-term bioassay for carcinogenicity, male and female mice of two hybrid strains, (C57BL/6 x C3H/Anf)F_1 and (C57BL/6 x AKR)F_1 were treated with atrazine for about 18 months. In this study, mice were given the maximum tolerated dose of 21.5 mg atrazine/kg by stomach tube from days 7 to 28 of age. After weaning at 28 days of age, atrazine was mixed in the diet at 82 ppm for the remainder of the experiment. Gross and microscopic examinations revealed no

significant increase in tumors when compared with the controls (Innes et al. 1969).

A preliminary study of the carcinogenic potential of the formulation Fogard S® (25 percent atrazine and 37.5 percent simazine as active ingredient) was reported by Donna et al. (1981). In this study limited, 25 Swiss albino mice weighing 25 grams each were given 13 subcutaneous injections of 0.005 mg Fogard S® at 3-day intervals for a total dose of 0.0065 mg active ingredient (0.0026 mg atrazine equivalent to 0.104 mg/kg). Fogard S® was administered intraperitoneally to a second group of mice. In both groups, the individual dosages were equivalent to 0.08 mg atrazine/kg body weight. The animals were observed for 7 months. Histological examinations were performed on all animals which died spontaneously or were sacrificed at the end of the experiment. Following subcutaneous treatment with Fogard S®, malignant lymphomas (cancer of the lymph nodes) were observed in three animals which died spontaneously. A peritoneal mesothelioma (a tumor arising from the cells of membranes lining the abdominal cavity) was observed in one sacrificed animal.

In the group treated intraperitoneally with Fogard S®, five animals were lost to cannibalism. Of the remaining animals, two spontaneous deaths were due to malignant lymphomas. No tumors were found in control animals. This preliminary study appears to indicate that Fogard S® has the potential to induce malignant lymphomas in mice although the small sample size and short observation period are not adequate for further evaluation of the study. The role of tumor induction by atrazine alone in this study is uncertain. In light of the long-term chronic feeding study by Innes et al. (1969), in which mice were given much larger (approximately 12.3 mg/kg vs 0.02 mg/kg) doses and observed for 18 months without any significant increases in tumor induction, the apparent tumorigenicity of Fogard S® is questionable.

2.5 Mutagenicity

Atrazine has been examined in a variety of microbial mutagenicity tests and generally gave negative results when tested in the presence or absence of metabolic activation systems prepared from animal tissues. However, when tested in the presence of extracts prepared from plants such as potato or Nicotiana, several systems gave positive mutagenicity results. Results of the various studies are summarized in Appendix E and Table 2-1.

Except for the report by Njagi and Gopalan (1980), all results were negative when atrazine was tested in the Salmonella typhimurium microbial mutagenicity test system in the presence or absence of an S-9 metabolic activation system or when tested in a host-mediated

assay (Andersen et al. 1972; Simmon et al. 1977; Loprieno and Adler 1980; and Seiler 1973). In the study by Njagi and Gopalan (1980), alachlor, containing 34 percent atrazine as active ingredient, was reported to be mutagenic for S. typhimurium strains TA 98, TA 1535, TA 1537 and TA 1978. Surprisingly, these results were obtained in the absence of an exogenous metabolic activation system. The study could not be critically evaluated because no numerical data were presented.

Plewa and Gentile (1976) reported that extracts of maize plants treated with atrazine were mutagenic for the yeast Saccharomyces cerevisiae. Atrazine was not mutagenic when applied directly to the yeast culture. Atrazine was not mutagenic to S. cerevisiae when tested in the presence of mammalian metabolism system (deBertoldi et al. 1978, 1980 and Marquardt 1980), in a host-mediation assay (Marquardt 1980), or when tested in the presence of potato microsomes (Loprieno and Adler 1980).

Solt and Neale (1980) reported that atrazine was mutagenic in a host-mediated system employing Escherichia coli. In this system mice were treated orally with the herbicide. Bacteria were then injected intravenously, reisolated several days later, and screened for resistance to antibiotics. Doses of atrazine, test conditions, and results of the tests were not further described. Previously, deBertoldi et al. (1978) reported that atrazine was nonmutagenic to E. coli in a host-mediated forward mutation assay measuring resistance to ampicillin.

Atrazine was mutagenic to Aspergillus nidulans in a forward mutation assay for resistance to 8-azaguanine and for mitotic crossing over when tested in the presence of plant microsomes, but nonmutagenic in a mitotic gene conversion assay (deBertoldi et al. 1978). In another study (Carere and Morpugo 1981), atrazine was positive for the induction of 8-azaguanine resistance and mitotic crossing over in A. nidulans only after cultures were treated with metabolities extracted from treated Nicotiana plants.

Loprieno and Adler (1980) reported that atrazine was mutagenic to the yeast Schizosaccharomyces pombe in forward mutation assays when tested in vitro in the presence of either potato microsomes or liver microsomes from mice treated orally with atrazine. The same authors reported that atrazine was mutagenic to Streptomyces coelicolor in a forward mutation assay for resistance to the antibiotic streptomycin when the bacterial culture was tested in the presence of potato microsomes.

Atrazine was not mutagenic to the E. coli bacteriophage T_4 or to rII mutants of the bacteriophage (Andersen et al. 1972).

Results with the fruit fly Drosophila melanogaster are equivocal. Murnik and Nash (1977) assayed atrazine for dominant lethal mutations, sex-linked recessive lethal mutations, chromosomal breakage, and nondisjunction or loss in Drosophila. A significant increase in the rate of dominant lethals was observed. The authors attributed this increase to a reduction in sperm transferred from treated males or to toxicity. X-linked recessive lethals occurred when atrazine was injected, but not when fed to the mice. They observed no significant increase in partial loss of the Y chromosome or sex chromosome disjunction.

In another study in D. melanogaster, atrazine was reported to be nonmutagenic when tested for recessive lethals. No details of the study were presented (Loprieno and Adler 1980).

Atrazine was tested in a number of mammalian cell systems. In a forward mutation assay for resistance to 6-thioguanine, atrazine was mutagenic when tested in the presence of potato microsomes (Loprieno and Adler 1980). When tested for the induction of chromosome aberrations in the presence or absence of metabolic activation, atrazine was nonmutagenic to Chinese hamster cells (Sobels et al. 1980) or CHO hamster cells (Loprieno and Adler 1980), but gave positive results with mouse bone marrow cells (Ehling 1980 and Loprieno et al. 1980). Equivocal results were reported for the induction of dominant lethal mutations in mice. Ehling (1980) reported positive results while Loprieno and Adler (1980) reported negative results. Atrazine gave negative results when tested for the induction of sister chromatid exchanges in CHO hamster cells but gave positive results for unscheduled DNA synthesis in EUE human cells (Loprieno and Adler 1980).

Several mutagenicity studies were performed in plants. Atrazine was shown to induce chromosomal aberrations in root tip cells of barley following the treatment of seeds or seedlings with solutions of the herbicide (Wuu and Grant 1966a and 1966b). Soaking or spraying barley seeds with a solution of atrazine induced chromosomal aberrations in the meiotic cells of barley plants (Wuu and Grant 1967). Maize plants were grown in the presence of atrazine to determine the effect on plant germ cells. Chou and Weber (1981) reported the induction of sister chromatid exchanges in maize after strain B37 inbred seedling roots were treated with atrazine at 10 and 20 ppm. The effect was due to the conversion of atrazine to a mutagenic metabolite by the plant. An increased frequency of reversion at the waxy locus was observed when pollen grains from tassels at the anthesis stage were removed and examined (Plewa and Gentile 1976).

3.0 Environmental Fate

Atrazine is a nonselective herbicide which can be used to control perennial grasses and broadleaf weeds, however, it may be toxic to nontarget plant species. It is persistent to moderately persistent in soil, is moderately persistent in water, and has a relatively low toxicity to most species of animals. It is absorbed primarily by plant roots from which it is readily translocated to all of the above-ground parts of the plant, with some accumulation at the margins of leaves. Atrazine is readily metabolized in plants by a variety of metabolic pathways. It is rapidly metabolized and does not tend to bioaccumulate in animals (Esser et al. 1975).

3.1 Bioaccumulation and Metabolism

Bioaccumulation and metabolism of atrazine in both animals and plants have been extensively reviewed by Esser et al. (1975). Plant metabolism of atrazine is also reviewed by Ghassemi et al. (1981) and by Ebert and Dumford (1976). A number of studies, reviewed in Section 3.1.2, examine the bioaccumulation and metabolism of atrazine in animals.

3.1.1 Plants

For the most part, studies of the uptake and metabolism of triazines (including atrazine), have been undertaken with plants grown in nutrient solution rather than in plants grown under normal field conditions (Esser et al. 1975). These studies indicate that the major pathway in plants for uptake of atrazine is through the roots, with both susceptible and resistant plants readily absorbing the compound. Atrazine applied to plant foliage does not penetrate readily, although the addition of an adjuvant, such as petroleum oil, tends to increase the rate of foliar penetration (Nalewaja and Adamczewski 1976). Studies with radiolabeled atrazine have shown that once the herbicide is absorbed into the plant roots, it is rapidly translocated by the xylem into the above-ground portions of the plant. It is then distributed throughout the plant with some tendency for accumulation in the leaf margins. The rates of uptake and translocation appear to be increased by factors such as higher temperature and lower relative humidity, which tend to increase the rate of transpiration and fluid movement within the plant. The increased movement of water thus facilitates movement of atrazine into, and through, the plant (Esser et al. 1975).

Phytotoxicity of herbicides is generally due to inhibition of, or interference with, one or more plant biochemical pathways. The triazines, including atrazine, act on a variety of biochemical

processes in plants, including photosynthesis, plant growth regulation, nitrogen metabolism, and nucleic acid metabolism (Esser et al. 1975). Ashton et al. (1977) found >90 percent inhibition of photosynthesis, 71 to 90 percent inhibition of RNA synthesis, 41 to 70 percent inhibition of lipid synthesis, and 20 to 40 percent inhibition of protein synthesis compared to controls in isolated bean leaf cells incubated in 10^{-4}M atrazine.

Atrazine interference with photosynthesis involves inhibition of the Hill reaction, the reaction in which oxygen is evolved from water in the presence of chloroplasts (Shimabukuro and Swanson 1969). The impairment of photosynthesis has several indirect effects, such as closing of stomata (openings through which gases pass) in the leaves as a result of increased carbon dioxide levels in the plant. The closed stomata in turn lead to a decreased rate of transpiration and consequent reduction in water uptake by the plant roots.

Although the exact biochemical events involved are unclear, a large amount of data indicate that atrazine (as well as other triazines) affects plant metabolism, particularly as it is governed by growth regulation hormones. This conclusion results from studies in which triazines (including atrazine), when applied at low rates, resulted in more vigorous and healthy plants than controls, yet at higher rates of application, resulted in growth inhibition. The enhancement and inhibition of plant growth at different levels of application has been shown to occur in a wide variety of plant species, including both atrazine resistant and susceptible plants.

Nitrogen metabolism is influenced by sublethal levels of triazine, resulting in increased protein fractions and dry matter of seeds from some treated plant species. Field and laboratory studies indicate that applications of triazines result in increased uptake of mineral nutrients, including nitrogen (when supplied as nitrates but not when supplied as ammonium). The increased uptake may be due to membrane changes facilitating more rapid uptake, or to larger roots with greater absorptive surface area in sublethally treated plants. Finally, there has been some consideration of the possible effect of triazines on nucleic acid metabolism, since nucleic acid metabolism is closely linked with growth and with protein metabolism. Experimental results are such that it is difficult to interpret the potential influence, if any, on plant nucleic acid metabolism.

The ability of any given plant species or variety to resist herbicidal activity of atrazine is directly related to its ability to degrade atrazine, hence those species or varieties which readily degrade atrazine to nonphytotoxic or less phytotoxic compounds are less susceptible to its herbicidal activity. In plants, at least six types of reactions by which atrazine can be biodegraded have been

identified, including hydrolysis, conjugation of atrazine with other molecules in the plant, removal of side chains from the atrazine molecule with liberation of carbon dioxide, modification of the side chain of the atrazine molecule by processes such as oxidation, replacement of amino groups (NH_2) by hydroxy (OH) substituents, and cleavage of the triazine ring structure with ultimate production of carbon dioxide (Esser et al. 1975). Studies with various chloro-triazines (of which atrazine is one) in living plants indicate that "hydroxytriazines" formed by hydrolysis of the parent triazine are the primary degradation products.

3.1.2 Animals

Atrazine has a low tendency to bioaccumulate in animals and, once absorbed by animals, it is rapidly metabolized to nontoxic metabolites that are readily excreted via the kidneys. Appendix F summarizes studies on the bioaccumulation and metabolism of atrazine. A detailed review of the metabolism of atrazine, as well as the other triazine herbicides, can be found in Esser et al. (1975).

Atrazine degradation in animals has been shown to occur by several pathways and has been postulated to occur by several others (Esser et al. 1975). These pathways include hydrolysis of the parent atrazine to hydroxyatrazine, conjugation (combining) of the parent herbicide with other organic metabolites in the animal, removal of the side chains and their further degradation to carbon dioxide, modifications to the side chains, and cleavage of the triazine ring.

In general, when atrazine is ingested by animals, it is degraded into intermediate metabolites that are readily excreted by the kidneys. When plant metabolites are fed to animals, they are further metabolized by the animals. Studies with ^{14}C-labeled triazines indicated rapid and complete elimination of radioactivity, primarily via urine. Generally, triazines are excreted very rapidly, with 50 percent of the applied dose eliminated within 12 to 26 hours in laboratory animals and 1 to 2 days in ruminants. When goats and cows are given single doses of triazine at a rate of 1 mg/kg, transient residues occur in the milk, with peak concentrations 8 to 24 hours after ingestion and rapid decline in residues to the limit of detection within 2 to 4 days (Esser et al. 1975).

Bioaccumulation studies, and studies of atrazine and metabolite residue levels in animal tissues (Appendix F), indicate generally low concentration factors for atrazine (Bathe et al. 1975; Gunkel and Streit 1980; and Gunkel 1981) as well as low residual levels of atrazine and metabolites in tissue (Khan and Foster 1976; Bathe et al. 1975; and Newton and Norris 1968 in U.S. Department of Energy 1983).

These factors, coupled with the very rapid excretion of atrazine and its metabolites (Foster and Khan 1976; Bakke et al. 1972a and 1972b; and Erickson et al. 1979), indicate that low levels of atrazine and its metabolites, if any at all, can be expected in most wildlife animals soon after atrazine application.

3.2 Soils

The fate and transport of atrazine in soil has been reviewed by a number of authors, including Esser et al. (1975) and Ghassemi et al. (1981). In addition, Volume 32 of Residue Reviews is a compilation of 14 papers on the fate and transport of triazines in the soil environment, some of which have been incorporated in this discussion.

Atrazine is moderately persistent (3 to 12 months) in soil compared to other herbicides, permitting full season control of vegetation (Ashton 1982). Atrazine has a relatively low solubility in water (Weed Science Society of America 1983), has a moderate mobility in silty clay loam (Helling 1970, 1971), and is relatively readily adsorbed onto soil particles, particularly in muck and clay soils with high organic matter content (Weed Science Society of America 1983). Chemical and microbial degradation are the primary means by which atrazine is lost from soil environments. In field situations, particularly in forest environments, photodegradation and volatilization should not account for any significant loss of atrazine (Ghassemi et al. 1981 and Weed Science Society of America 1983).

The persistence of atrazine has been studied in a wide variety of soil types. The relative persistence of atrazine under most conditions provides residual activity that makes it particularly useful for weed control and soil sterilization applications. At the same time, however, this persistence can result in injury to plants such as sensitive rotational crops (Sheets 1970 and Mullison 1970). In an early review of soil persistence of triazines, Sheets (1970) reported that most studies found atrazine to be less persistent than simazine and that it was more readily lost during warmer weather and heavy precipitation than during cold weather and under dry conditions. He also reported that atrazine persistence increases with depth to about 24 inches, below which loss may increase in some soils, probably due to dilution. Table 3-1 summarizes major studies on atrazine persistence in soil conducted in the United Kingdom and in North America, mostly during the past 10 years. These studies confirm that atrazine is moderately persistent (with residual activity often lasting up to a year) and that atrazine tends to remain in the top layers of the soil. Of particular interest in Table 3-1 are the studies by Khan and Saidak (1981 in Smith 1982) and by Jones et al. 1982, in which hydroxyatrazine, the primary soil metabolite, was found to have a relatively long persistence in soil.

Table 3-1

Summary of Soil Persistence Studies with Atrazine

Formulation and Application Rates	Effects	Comments	References
Atrazine, 2 to 10 lb/acre (2.24 to 22.4 kg/ha) applied in field in June (loam, Quebec)	After 10 weeks, 18 to 44% remained and after 52 weeks, 6 to 8% remained.	Depth of 0 to 2 inches. Slight leaching observed.	Birk and Readhouse 1964 in Smith 1982
Atrazine,1 to 3 lb/acre (1.12 to 3.36 kg/ha) applied in field in May to June (clay loam, Ontario)	After 22 weeks, about 15% remained, and after 52 weeks, less than 10% remained.	Depth of 0 to 2 inches.	Sirons et al. 1973 in Smith
Atrazine, 0.25 to 2 lb/acre (0.28 to 2.24 kg/ha) applied in field in May to June (clay loam, Ontario)	10 to 20% remained after 52 weeks	Depth of 0 to 6 inches.	Marriage et al. 1978 in Smith 1982
Atrazine, 1.5 lb/acre (1.68 kg/ha) applied in field in May to June (clay loam, Ontario)	After 7 weeks, 48 to 53% remained, after 18 weeks, 23 to 28%, and after 52 weeks, about 10% remained.	Depth of 0 to 6 inches	Khan et al. 1981 in Smith 1982
Atrazine applied in May at 1.25 to 2 lb/acre (1.4 to 2.24 kg/ha) to corn plots that had been treated 20 consecutive years (sandy loam, Ontario)	In October, 102 ppb atrazine and 378 ppb hydroxyatrazine detected. In following May (1 year) 55 ppb atrazine and 296 ppb hydroxyatrazine detected.	Depth of 0 to 6 inches. Other herbicides used for weed control during 20-year pretreatment.	Khan and Saidak 1981 in Smith 1982
Atrazine applied in late May at 4 lb/acre (4.5 kg/ha) to peach orchard that had been treated 9 consecutive years (sandy loam, Ontario)	Maximum residues during October sampling (last 3 years) were 0.36 lb/acre (0.4 kg/ha) for atrazine.	Depth of 0 to 6 inches. Other herbicides used for weed control. Trees removed 3 years before end of reported study.	Marriage et al. 1975
Atrazine, 3 lb/acre (3.36 kg/ha) applied in sandy loam (Ireland)	After 6 months, 0.57 ppm atrazine in soil.	Depth of 0 to 3 inches. No post harvest cultivation.	Mitchell 1973

Table 3-1 (Continued)

Formulation and Application Rates	Effects	Comments	References
Atrazine, 4 lb/acre (4.48 kg/ha) two applications in medium loam (Ireland)	After 9 months, 0.89 ppm atrazine in soil.	Depth of 0 to 3 inches. No post harvest cultivation.	Mitchell 1973
Atrazine, 4 lb/acre (4.48 kg/ha) applied in clay (Ireland)	After 9 months, 0.15 ppm atrazine in soil.	Depth of 0 to 3 inches. No post harvest cultivation.	
Atrazine, 3.2 to 25.6 lb/acre (3.6 to 28.7 kg/ha) applied per year for 9 years (California)	Nine months after 4th application, residues of 0.81 and 10.8 lb/acre were measured (for soils treated with 3.2 and 25.6 lb/acre/year, respectively); 21 months after 9th application total residues were 0.47 and 3.46 lb/acre, 70% breakdown per year.	In 0 to 6 inch layer 21 monthly after 9th application, 0.13 and 1.40 ppm atrazine (for soils treated with 3.2 and 25.6 lb/acre/year respectively). In 6 to 12 inch layer, 0.06 to 0.39 ppm and 0.06 to 0.33 ppm in 12 to 18 inch layer.	Leonard et al. 1975
Atrazine, 3.3 to 4.1 lb/acre (3.66 to 4.54 kg/ha) applied in May (Eastern Ohio)	Degraded through 3 to 4 half-lives during the 160-day growing season.	Under both no-till and conventional management.	Nearpass et al. 1978
Atrazine, 1.8 lb/acre (2 kg/ha) applied in late April to coarse sandy loam (U.K.)	Percent atrazine recovered in sample cores were 94 $\pm$ 11% at 3 days; 84 $\pm$ 14.9% at 14 days; 76 $\pm$ 9.7% at 27 days; 51 $\pm$ 7.7% at 57 days; 34 $\pm$ 4.3% at 83 days and 19 $\pm$ 2.1% at 111 days.	Cores taken to a depth of 6 inches.	Nicholls et al. 1982
Atrazine, 1 lb/acre (0.89 kg/ha)	Persistence greater than 60 weeks.	Further details not given in review.	Hocombe et al. 1966 in Williams 1970
Atrazine, 1.6 lb/acre (1.8 kg/ha) in desert cropland soils, (Arizona) and incorporated by disk	Atrazine reduced soybean growth (fresh weight) 29 months after application to soil. At one site (luma) stands of cabbage and sugarbeets were reduced at 24 months and at Mesa, stands of alfalfa, barley, sugarbeets and lettuce.	—[a]	Hamilton and Arle 1972

Table 3-1 (Concluded)

Formulation and Application Rates	Effects	Comments	References
Atrazine, incorporated in soil at 6, 16 and 36-inch depths at 1 and 2; 1; and 0.5 and 1 ppm, respectively, in silty clay loam and sandy loam (Nebraska)	After 5 months, differences in aerobic degradation observed with depth-more degradation near surface in warmer, higher organic matter and micro-organism populaus upper layers. At 17 months, all atrazine gone from 6 and 16-inch layers, but even at 41 months, phyto-toxic activity remained at 36 inches.	Both aerobic and anaerobic degradation patterns were similar, suggestive of strict chemical conversion or presence of faculta-tive microorganisms.	Lavy et al. 1973
Atrazine, ^{14}C-labeled applied to sandy loam and to silty loam to give concentrations of 6.4 and 5.0 ppm with soils kept in flasks held in sun-light and ambient temperature (Chesapeake Bay)	Atrazine half-lives were cal-culated to be 330 and 385 days for the sandy loam and silty loam respectively. Hydroxy-atrazine was the major short-term metabolite.	Sandy loam soil (Sassfras) held at 60% water holding capacity (17% water by weight). Silty loam soil (Mattapex) held at 100% water holding capacity (50% water by weight).	Jones et al. 1982
Atrazine, (80% wettable powder) 0.0, 0.5, 1.0, 2, 4, 6, and 8 lb a.i./acre (0.0, 0.6, 1.1, 2.2, 4.5, 6.7 and 9.0 kg/ha) applied in mid-May in field to silty clay loam (Pennsylvania)	Average of 67.9% in soil 1 month after application; 3 months later, 21.4%; 11 months later, 15.9%; 16 months later, 5.4%.	--	Hall et al. 1972
Atrazine applied at 2 lb and 4 lb/acre (2.28 and 4.48 kg/ha) applied to loamy sand soil in late May after planting with corn (Georgia)	Persistence of atrazine did not vary with application rate; concentration in top 4 inches of soil increased with appli-cation rate but not concentra-tions at lower soil levels. Atrazine persisted in surface 4 inches for over 4 months, but could not be detected at lower levels after 2 months.	--	Rohde et al. 1081

[a]Data not available or not applicable.

Helling (1970, 1971) compared atrazine to 39 other herbicides, including other triazines, in the laboratory and demonstrated that, in silty clay loam, atrazine was only moderately mobile. Lavy (1970) compared movement of atrazine, propazine, and simazine in soil due to diffusion and leaching and reported that, although atrazine diffused faster than the other two triazines studied, it occurred at such a slow rate that diffusion could not be an important mechanism of vertical movement of triazines in soil. He therefore concluded that under most circumstances, leaching with water would be the more important means of atrazine transport in soil. Dao (1979) concluded that diffusion as a process was only a small contributor to the overall movement of atrazine in the soil, with diffusion rates in saturated soils on the order of 0.2 to 1.1 X 10^{-7} cm^2/sec. The movement of atrazine by leaching in a saturated soil column was reduced by a reduction in the rate of water flow through the column. Rao and Davidson (1979) and Davidson et al. (1980) report that atrazine mobility was greater for higher concentrations than for lower concentrations in a silty clay loam, a sandy loam, and in a fine sand in water-saturated soil columns in the laboratory. The actual proportional amount of soil-applied atrazine in field situations that is leached to soil depths (28 to 64 inches; 70 to 163 cm) is quite low, with 0.13 to 1.1 percent of the applied atrazine recovered in tile drainage water from cornfields (Von Stryk and Bolton 1977 and Muir and Baker 1978, both in Smith 1982).

A number of studies have examined the potential for runoff loss of soil-applied atrazine. Foy and Hiranpradit (1977) compared runoff losses of atrazine from "no-till" and "conventionally-planted" corn plots treated with 4 lb/acre (4.5 kg/ha) sprayed pre-emergence. Atrazine runoff from these plots during a single sprinkler irrigation resulted in runoff loss of 0.5 and 1.7 percent of the total applied atrazine from the no-till and conventionally-planted plots, respectively. During rainfall, 3.9 percent of the total applied atrazine was collected in runoff from conventionally-planted plots, while no-runoff occurred from the no-till plots. For small (1.9 to 3.8 acre) watersheds of loessial soil in Iowa, planted in corn and sprayed with at a rate of 3 lb/acre (3.36 kg/ha) in early May, up to 0.448 lb/acre (15 percent of total applied atrazine) was in runoff from a rainfall event 7 days after atrazine application. Of this amount, 0.339 lb/acre was in the runoff water and 0.109 lb/acre was in the runoff sediments (Ritter et al. 1974). The amount of atrazine in runoff generally decreased when the time between application and precipitation increased. Gaynor and Volk (1981) compared runoff losses of atrazine from a silt loam soil either unlimed or treated with lime. Atrazine (80 percent wettable powder formulation) was applied at a rate of 3.2 lb a.i./acre (3.6 kg/ha) and treated with 5.5 to 7.4 cm of water 12 hours later. The runoff water and sediment for unlimed soil contained 0.3 percent of the total applied atrazine,

of which all of the total atrazine was in the water. In the 8-hour watering, no atrazine was detected in runoff from limed soil. When the unlimed soil plots were watered (6.9 to 7.3 cm) 8 days after atrazine application, 1.6 percent of the atrazine applied was in runoff water and 0.05 percent was found in sediments. For limed soil, less than 0.1 percent was found in water and less than 0.01 percent in sediments. Lime application appeared to have improved soil structure, resulting in reduced runoff and herbicide loss compared to the unlimed soil. When the amounts of residual atrazine in both soils were assayed at 10 to 12 days post-application, 25 percent was recovered from unlimed soils (12 days) compared to 74 percent from limed soils (10 days). The low recovery from unlimed soils is probably due to chemical degradation to hydroxyatrazine. In a field study with pre-emergent application of atrazine to corn at rates up to 8 lb a.i./acre (up to 9 kg/ha) in a silty clay loam, Hall et al. (1972) found that, during the year of application, average losses for all rates of application in runoff water and soil sediment equaled 2.4 percent and 0.16 percent of the total amount of atrazine applied, respectively. A wettable powder (80 percent active ingredient) formulation of atrazine (AAtrex®) was surface applied and incorporated into coastal plain soils (sandy loam and sandy clay loam) at rates of 1.5 and 3.0 lb a.i./acre (1.68 and 3.36 kg/ha) and then followed, 1 hour later, by a simulated rainfall equivalent to 5 inches (13 cm) in 2 hours to represent a 100-year frequency storm. Average atrazine losses in runoff were 10.7 percent (6.4 to 13.3 percent), most of which occurred during the first 40 to 50 minutes of rainfall. In terms of the absolute amount of herbicide loss, 70 to 80 percent was in runoff water although actual concentrations of atrazine were greater in runoff sediment, probably due to adsorption of atrazine to clay and silt particles. Atrazine losses of 7.3 percent were reported in the runoff of a 10-year frequency storm (2.5 inches rainfall per hour) occurring 96 hours after atrazine was applied to fallow plots of soil with a 6.5 percent slope (White et al. 1967 in Ghassemi et al. 1981). After application of 2 and 4 lb atrazine/acre (2.24 and 4.48 kg/ha) to a loamy sand soil, Rohde et al. (1981) could not detect significant amounts of atrazine in surface runoff after 26 days nor in subsurface flow at any time. Total losses of atrazine over a 2 year period ranged from 0.22 to 2.24 percent of that applied.

Atrazine is readily adsorbed to soil particles, with a greater tendency to adsorb on muck or clay soils than on soils with a low clay and organic matter content. This tendency for atrazine to adsorb to soils serves to limit downward movement of the herbicide to deeper soil layers by leaching and reduces uptake by crops and weeds because there is less atrazine available for soil solution as a result of adsorption (Weed Science Society of America 1983 and Williams 1970). Since forest soils have large amounts of organic matter, there should be a strong tendency for atrazine to adsorb

to soil particles and these soils should offer a high degree of resistance to leaching of atrazine. In addition, soil organic matter appears to be the most active soil component involved in reducing the phytotoxic effects of triazine herbicides (Hayes 1970 and Adams and Pritchard 1977). Dao (1979) found atrazine adsorption to increase with decreased soil moisture, to increase with an increase in electrolyte (salt) levels in soil, and to increase with an increase in temperature. Scott and Phillips (1972) demonstrated an increase in soil diffusion of atrazine with increasing amounts of soil water, reflecting decreased adsorption to soil particles at higher levels of water content.

Microbial degradation of atrazine has been variously reviewed by Esser et al. (1975), Ghassemi et al. (1981), Kaufman and Kearney (1970) and Paris and Lewis (1973). There is a large body of literature indicating that soil microorganisms are capable of metabolizing triazines in general, and atrazine in particular, as a source of energy. Kaufman and Kearney (1970) list 4 species of bacteria and 21 fungi that have been shown effective in metabolizing atrazine. Srirama Raju and Rangaswami (1972) reported that some bacteria and fungi were resistant to both atrazine and simazine at herbicide concentrations as high as 7,500 ppm. The three major routes for microbial degradation of atrazine are dealkylation (removal of the side chains and evolution of carbon dioxide), hydrolysis to form the metabolite hydroxyatrazine, and cleavage of the triazine ring (Ghassemi et al. 1981). As one would expect, the highest rates of atrazine inactivation are generally found under those conditions that are favorable for microbial growth, such as warm temperatures, high moisture, and high organic matter content (Esser et al. 1975). In a laboratory study with atrazine incubated in soils taken from different depths, Roeth et al. (1969) demonstrated that atrazine degraded 2 to 3 times more rapidly in topsoils than in subsoils, that each 10°C temperature increment (from 15°C to 35°C) resulted in a two- to threefold increase in degradation rate, and increased soil moisture content (from 0.4 to 0.8 field capacity) resulted in $^{14}CO_2$ evolution from ^{14}C atrazine-treated soil to increase from 0 to 6 times.

Chemical degradation of atrazine has been discussed in Section 1.2. Studies with sterilized soil indicate that hydrolysis to form hydroxyatrazine is a major route of nonbiological degradation of atrazine. Organic matter in the soil, such as humic acid and soil colloids, catalyse this hydrolytic degradation at low pH, while other soil compounds, such as iron and aluminum ions, promote replacement of the chlorine on the triazine ring by the OH^- ion (Khan 1978 in Ghassemi et al. 1981).

Volatilization and photodegradation of atrazine is reviewed in Jordan et al. (1970) and Ghassemi et al. (1981) and is briefly discussed in Section 1.2 of this Background Statement. Atrazine has

a relatively low vapor pressure. It is more readily volatilized from the surface of foliage than from the surface of soil. Atrazine volatilization from soil is reduced by soil moisture (Kearney et al. 1964 in Ghassemi et al. 1981). Atrazine has been demonstrated to photodegrade when exposed to ultraviolet radiation in sunlight when applied to soil surfaces (Jordan et al. 1970). Photodecomposition occurs to some extent in field situations if high temperatures and prolonged sunlight follow application of atrazine before precipitation (Weed Sciences Society of America 1983). However, the mechanism and products of photodegradation in soil have not been investigated to the extent that they are clearly understood. In forest soils, increased moisture and reduced sunlight should reduce further the small potential for atrazine volatilization and photodegradation.

3.3 Water

Atrazine is only slightly soluble in water and its low solubility, coupled with a tendency to adsorb to soil particles, results in only relatively small amounts of atrazine in runoff water. Atrazine degrades slowly in water, thus dilution, transport, and metabolism by aquatic plants, algae, and bacteria are the prime means by which atrazine concentrations are reduced in aquatic environments.

The aesthetic qualities of water containing atrazine were examined by Martynyuk and Gzhegotskii (1967 in Mullison 1970). Water containing 0.1 to 5.0 ppm atrazine did not differ from control samples in terms of its sensory qualities, even when heated to 140 to 158°F (60 to 70 C) to increase vaporization of noxious substances. Higher concentrations of atrazine (50 ppm or greater) resulted in greatly altered sensory qualities of the water. When bottled ground water containing 600 to 1,000 ppm atrazine was stored at 6 to 22°C for as long as 6 months, no decrease in atrazine concentration was observed.

Atrazine is transported in water either in surface runoff following precipitation events, in groundwater flow, or in streamflow. Atrazine transport in surface runoff has been discussed in Section 3.2 of this background statement. Under most environmental conditions, only relatively small amounts of atrazine should be transported in runoff water. Such transport would occur primarily during rainstorm events within 1 or 2 days after atrazine application. Schneider et al. (1971) evaluated the potential for atrazine contamination of groundwater aquifers by pumping water containing 1.28 ppm atrazine into the Ogallala aquifer at an average rate of 360 gallons per minute for 10 days. Atrazine could be detected in water pumped from a well 66 feet from injection after 10 days of pumping, but not in a well 150 feet from the injection well. When groundwater was pumped out of the injection well (starting 10 days after injection stopped) at a rate of 500 gallons per minute for 9 days and 480 gallons per

minute for 3 days, essentially all of the injected atrazine was recovered. After 189 hours of pumping, atrazine concentrations in the well water were 0.25 ppm. The authors concluded that water from a dual-purpose well (aquifer recharge and pumping) contaminated with a herbicide such as atrazine could be used for irrigation of atrazine tolerant crops, and the injected contaminated water would not move very far in the aquifer if it were pumped out relatively soon. Atrazine transport in streamflow could occur mainly as a result of surface runoff of atrazine from soils or direct application of atrazine to watercourses. Such transport and subsequent dilution would result in a relatively rapid decrease in atrazine concentrations in water. Atrazine measurements in water are discussed below in this Section.

Degradation of atrazine has been studied in marine and estuarine systems and in aquatic soils. Jones et al. (1982) compared persistence of atrazine in estuarine water and sediments under both aerobic and low oxygen conditions in a laboratory environment. They reported a relatively rapid loss of atrazine in the water (half-life of 3 to 12 days) and in the estuarine sediment (half-life of 15 to 20 days). The major short-term metabolite was hydroxyatrazine and it appeared that decreased oxygen levels had little effect on atrazine degradation.

Several studies have measured residues of atrazine in streams as a result of normal agricultural activities or as a result of experimentation. Roberts et al. (1979) monitored atrazine and its residues in a watershed in southwestern Ontario in which approximately 25.6 to 26.8 percent of the total area (4,500 hectares) had been planted in corn and treated with atrazine at average rates of 0.9 to 1.04 lb/acre (1.02 to 1.16 kg/ha) in 1973 and 1974, respectively. Of 360 water samples within the watershed taken from May 1973 through February 1975, 320 (89 percent) contained atrazine. Of 235 samples taken from May 1974 through February, 121 (34 percent) contained desethylatrazine, an atrazine metabolite. The highest level of atrazine in creek water, 34.7 ppb, was observed in mid-June. Of the water samples taken at the mouth of the creek draining the watershed, however, maximum levels of 3.3 ppb atrazine were detected in July. Desethylatrazine levels rarely exceeded 1 ppb in this watershed, however. Frank (1981) monitored atrazine residues in the water of two agricultural water-sheds in Ontario encompassing a total of 1,079,000 hectares on which an estimated total of 209,800 kg of atrazine were applied in 1975. Maximum concentrations of 2 ppb (in June 1975) and 1.5 ppb (in July 1976) were measured at the mouths of the rivers draining the two watersheds. Based upon masurements of monthly flow volume and atrazine concentrations at the river mouths, it was estimated that less than 0.6 percent of the total atrazine applied to the two water-sheds was in the discharge at the river

mouths. Frank and Sirons (1979 in Norris et al. 1983) found atrazine or its metabolites in 80 percent of streams in 11 small agricultural watersheds (average size of 4,279 ha). Average concentrations of residues were 1.4 ppb with a peak concentration of 32 ppb. Approximately 62 percent of the atrazine discharge from these watersheds was associated with storm runoff. Richard et al. (1975) monitored atrazine residues during the 1974 growing season and found atrazine residue levels ranging from 0.05 ppb to 42 ppb in surface waters and residues of 0.480 ppb to below detectable limits in finished city water supplies in 12 Iowa cities. The highest concentrations of atrazine in surface waters were generally observed in June, and the highest concentrations in finished waters were observed in July. Atrazine residues were monitored in July 1977 in the Black Creek watershed, an 18.7 square mile (48.5 sq. km) watershed in Indiana (Dudley and Karr 1980). In 14 sediment samples, 7 water samples, and 18 fish samples, no atrazine residues were detected at a lower limit of detection of 0.1 ppm. Hoermann et al. (1979) monitored residues of triazines, including atrazine, at 17 sites along 9 rivers in central Europe from the spring of 1976 through the fall, 1977. Of a total of 118 samples, 59 percent contained less than 0.4 ppb atrazine, 24 percent contained 0.4 to 1.0 ppb, 15 percent contained 1.1 to 10 ppb, and only 2 percent contained more than 10 ppb atrazine. As with U.S. studies, peak residue levels occurred in June.

Atrazine residue levels have been monitored in agricultural runoff, in irrigation tailwater pits, and in irrigation ditches to determine the potential for residual toxic effects. Ritter et al. (1974) measured concentrations of atrazine in runoff water between 1.17 and 4.91 ppm during a storm 7 days after application of atrazine (wettable powder) at a rate of 2 lb/acre (2.24 kg/ha) to a surface-contoured watershed planted in corn. Maximum atrazine residues measured in water samples from tailwater pits used to collect irrigation runoff in Kansas were 1,074.1 ppb and residues persisted throughout the year (Kadoum and Mock 1978). No residues were detected in irrigation ditches and basins that had been sprayed with atrazine when dry and then subsequently filled twice with water (Smith et al. 1975 in Norris et al. 1983).

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to atrazine during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved atrazine. Calculations of exposure to atrazine in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to atrazine are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Atrazine Application

Available application data for atrazine use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to atrazine differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of atrazine in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Atrazine is readily adsorbed to soil particles. It has low water solubility and moderate soil mobility. It is primarily degraded by chemical and microbial processes. Atrazine is moderately persistent in soil with residual activity often lasting up to a year. The potential for incidental exposure of humans and wildlife to atrazine is moderate.

4.2 Exposure

Estimates of potential exposure to atrazine are based on aerial as well as ground application. The maximum aerial application rate for atrazine (see Table 1-1) is 5.6 lb a.i./acre, while the compound is applied from the ground (ground foliar) at rates as high as 8.0 lb a.i./acre for spot broadcast applications. In both aerial

Table 4-1

USDA Forest Service Application Data for Atrazine (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Atrazine (34)	Ground Foliar	6.7	228	4.9	9.4	6.6	10.6	Timber (28) Wildlife (2) Nursery (1) Research (1) Noxious Weeds (1) Engineering (1)

Source: Gross 1983

application and ground foliar application, atrazine is applied as a liquid. Calculations in Table 4-2 that are based on aerial application (e.g., observer dose, wildlife dermal exposures, and water concentrations) assume an application rate of 5.6 lb a.i./acre Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose and wildlife oral exposures) assume an application rate of 8.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of atrazine and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental dose and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of atrazine to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for atrazine are based on exposures on a per pound per acre application rate multiplied by 5.6 lb/acre for observers and 8.0 lb/acre for backpack sprayers. In the case of the aerial spray observer, dose estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent.

At 5.6 lb a.i./acre aerial application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.224 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 8.0 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.32 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Atrazine

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg[b]		
Observer	up to 0.224 mg/kg		
Backpack Sprayer	0.32 mg/kg		
Environmental Exposure			
Fish	0.056 ppm (Immersion)[c]		
Rabbits	2.262 mg/kg	Negligible	8.40 mg/kg
Deer	0.566 mg/kg	Negligible	11.52 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.0056 mg/kg (2 days)
(Rabbits)	NA	NA	0.038 mg/kg (2 days)
(Deer)	NA	NA	0.047 mg/kg (2 days)
(Water)	NA	NA	0.0022 mg/kg (1 day)
(Berries)	NA	NA	0.0056 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0028 mg/kg (1 day)
Toxicity Summary	LD_{50} = 9,300± 990 mg/kg in rats	1.6 ppm a.i. dust exposure caused no toxic effect in rats	Acute oral LD_{50}'s ≥1,400 mg/kg in rats and mice. Chronic feeding of 100 ppm to rats for 2 years caused no adverse effects.
	Most studies, LC_{50} >5 ppm for fish		

[a]Assumes aerial application at 5.6 lb active ingredient per acre and ground foliar application at 8.0 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure.

[c]Fish exposure results from immersion in water containing atrazine.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by atrazine application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of forest applicators. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 5.6 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 2.262 mg/kg (0.404 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.566 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of atrazine vapor would be negligible since the vapor pressure of atrazine is negligible (Weed Science Society of America, 1983). Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of atrazine in water, calculated as:

Water concentration: 0.056 ppm (0.01 ppm at 1.0 lb/acre aerial application rate)

This concentration of atrazine represents the estimated maximum concentration immediately after spraying. Due to the low potential for leaching of atrazine from soils, only negligible amounts of the herbicide would be expected to be carried into streams and ponds, even during rainfall soon after application.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of atrazine by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 8.0 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 11.52 mg/kg (1.44 mg/kg at 1 lb/acre application)

Rabbit: 8.40 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of atrazine in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.056 ppm
- bioconcentration: 5.0 (actual values reported in Appendix F generally range from 2 to 3).

Fish tissue concentration: 0.28 ppm (0.28 mg/kg)

Deer and rabbit tissue concentrations include atrazine accumulated from both oral and dermal exposure. Tissue concentration estimates

for deer and rabbit assume that dermally applied atrazine is absorbed at a rate of 10 percent and that 10 percent of the atrazine ingested by deer and rabbits is assimilated into muscle tissue. Data presented in Appendix F indicate that only a small amount of ingested atrazine is found in animal muscle tissue. Maximum residues of atrazine in tissues from deer collected an atrazine-treated forest lands were 0.076 ppm (Newton and Norris 1968 in U.S. Department of Energy 1983). It is also assumed that oral exposure of deer and rabbit to atrazine lasts only for 2 days due to the moderate persistence of atrazine. Deer tissue concentrations of atrazine are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.566 mg/kg (dermal exposure) x 0.10 = 0.0566 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 11.52 mg/kg/day (oral exposure) x 0.10 x 2 days = 2.304 mg/kg.

Total Deer Tissue concentration = 0.0566 mg/kg + 2.304 mg/kg = 2.3606 mg/kg (2.361 ppm).

Rabbit tissue concentrations of atrazine are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 2.262 mg/kg (dermal exposure) x 0.10 = 0.2262 mg/kg.

Oral Exposure contribution assumes 10 percent absorption of two-days feeding, or 8.4 mg/kg/day (oral exposure) x 0.10 x 2 days = 1.680 mg/kg.

Total Rabbit Tissue Concentration = 0.2262 mg/kg + 1.680 mg/kg = 1.9062 mg/kg (1.906 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to atrazine from eating meat are derived:

Fish meat: 0.28 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0056 mg/kg

Deer meat: 2.361 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0472 mg/kg

Rabbit meat: 1.906 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0381 mg/kg

Water and plant consumption by man would result in the following oral exposures to atrazine based on assumptions stated in the introductory chapter of the Herbicide Background Statements at an application rate of 5.6 lb a.i./acre (aerial):

Water: 0.0022 mg/kg (0.0004 mg/kg at 1 lb/acre application)

Berries: 0.0056 mg/kg (0.001 mg/kg at 1 lb/acre application)

Mushrooms: 0.0028 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of atrazine to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rats, rat inhalation results, rodent acute oral LD_{50}'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels anticipated for Forest Service applications.

Small mammal studies have shown that atrazine applied to the eye is "irritating" and that skin irritation tests produced some erythema. Fosgard S®, containing 25 percent atrazine, appeared to increase the incidence of lymphomas in mice in a preliminary study. However, a chronic lifetime study in mice with atrazine failed to show any carcinogenic activity, although much higher doses were employed. Atrazine increased the incidence of malignant lymphomas in one study in mice and caused no teratogenic effects in studies in rats, mice or sheep. It was non-mutagenic in most *in vitro* test systems.

These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from atrazine use, if proper care is taken during application.

Appendix A

Summary of Atrazine Toxicity Data For
Invertebrates and Microorganisms

Appendix A

Summary of Atrazine Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects[a]	Comments	References
Atrazine	Midge Larvae (Chironomus tentans; Insecta)	Surface absorption, static bioassay (acute toxicity)	48 hour	LC_{50} = 0.72 ppm (0.36 to 1.44 ppm), mortality observed at 0.5 ppm.	68 ± 1.8°F (20 ± 1°C)	Macek et al. 1976
Atrazine	Midge larvae (Chironomus tentans; Insecta)	Surface absorption, static bioassay (chronic toxicity)	2 generations	Continuous exposure to measured concentrations of 0.23 ppm or more through 2 generations resulted in reduced hatching success, increased larval mortality, retarded development, reduced rate of pupation and emergence. No adverse effect observed at 0.11 ppm.	—[b]	Macek et al. 1976
Atrazine	Mosquito (Culex restuans; Insecta)	Surface absorption, static bioassay	18 hours	LC_{50} 200 ppm for animals stressed with 0.4% methanol in 25 ml test system.	--	Bowman et al. 1981
Atrazine	German cockroach (Blatella germanica; Insecta)	Ingestion	—	A negligible effect on growth and reproduction through 2 generations was observed in animals fed 1,000 ppm atrazine in diet.	--	Riviere 1976
Atrazine	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption, static bioassay	18 hours	LC_{50} = 0.6 ppm (0.22 to 14.44 ppm) for animals stressed with 0.4% methanol in 25 ml test system.	--	Bowman et al. 1981
Atrazine (Atrazin® 99.2%)	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption, media exchanged daily	28 days and entire lifespan	Longevity was not affected by concentrations up to 10 ppm, but was reduced in animals exposed to 20 ppm. Reproduction (number of young per animal) was reduced at 1 ppm compared to control.	Use of ethanol (0.5%) as a solvent significantly reduced longevity and reproduction. A synergistic effect observed between atrazine and ethanol.	Schober and Lampert 1976, 1977

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Cladoceran (Daphnia magna; Crustacea)	Surface absorption; static bioassay	48 hours	LC_{50} = 6.9 ppm (5.2 to 8.1 ppm), mortality observed at 3.0 ppm.	68 ± 1.8°F (20 ± 1°C)	Macek et al. 1976
	Amphipod (Gammarus fasciatus; Crustacea)	(acute toxicity)	48 hours	LC_{50} = 5.7 ppm (3.6 to 8.0 ppm), mortality observed at 2.4 ppm.		
Atrazine	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay (chronic toxicity)	21 days	Continuous exposure to mean measured atrazine concentrations up to 1.15 ppm did not adversely affect survival through three generations. Mean number of young per female during first generation exposure was significantly reduced.	Data indicate that D. magna reproduction is more sensitive to atrazine than is survival.	Macek et al. 1976
Atrazine	Amphipod (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay (chronic toxicity)	30 days to 17 weeks	Survival of individual exposed continuously for 30 days to 0.94 ppm was reduced. No reduction observed in survival of individuals exposed to 0.49 ppm for 119 days. Reproductive effects and impaired survival of offspring observed at concentrations as low as 0.14 ppm.	--	Macek et al. 1976
Atrazine	Scud (Hyalella azteca; Crustacea)	Surface absorption, static bioassay	18 hours	LC_{50} = 8.8 ppm (7.2 to 18.64 ppm) for animals stressed with 0.5% methanol in 25 ml test systems.	--	Bowman et al. 1981
AAtrex® (80% wettable powder)	Fiddler crab (Uca pugnax; Crustacea)	Surface absorption in field enclosures and in greenhouse microhabitats.	10 weeks	Crabs exposed to a single application of 0, 100, 1,000 and 10,000 ppm a.i. Treatment at 10,000 ppm resulted in decreased survival in both field and greenhouse treatments.	--	Plumley et al. 1980

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
AAtrex® (80% wettable powder)	Fiddler crab (Uca pugnax; Crustacea)	Surface absorption in laboratory containers	8 weeks	Crabs exposed to 100 ml of 0, 1, 100, or 1,000 ppm a.i. suspension. In August, 1,000 ppm either killed crabs or eliminated their escape response. Adverse effects noted at 100 ppm, with severity a function of crab size and sex. With new crabs in subsequent tests, effects diminished so that in November, no effects observed at 1,000 ppm. Following August, tests confirmed seasonality of response with adverse effects similar to initial ones.	--	Plumley et al. 1980
AAtrex® (80% wettable powder)	Fiddler crab (Uca pugnax; Crustacea)	Oral, chronic exposure	20 days	No adverse effects in crabs fed detritus soaked with a 22 ppm atrazine solution.	--	Plumley et al. 1980
Atrazine	Cladoceran (Daphnia magna; Curstacea)	Surface absorption	48 hours	LC_{50} = 3.6 ppm	--	U.S. Department of the Interior 1968 in Pimentel 1971
Atrazine	Brown shrimp (Penaeus aztecus; Crustacea)	Surface absorption	48 hours	Thirty percent mortality or paralysis in shrimp exposed to 1 ppm.	--	Butler 1965
Atrazine	Shore crab (Carcinus maenus; Crustacea)	--	--	LC_{50} >100 ppm	--	Portmann and Wilson 1971 in Ghassemi et al. 1981
Atrazine	Clam (Pelecypod Mollusc)	Exposed in pond enclosures, surface absorption	--	Clams reduced to 1/8 of original number after application of atrazine at dosages of 0.5 to 2.0 ppm.	--	Walker 1962 in Pimentel 1971

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Snails (Gastropod Mollusc)	Exposed in pond enclosures, surface absorption	—	Snail population increased nearly 4 times after application of atrazine at dosages of 0.5 to 2.0 ppm.	—	Walker 1962 in Pimentel 1971
Atrazine	Eastern oyster (*Crassostrea virginica*; Pelecypod Mollusc)	Surface absorption	96 hours	No noticeable effect on shell growth in oysters exposed to 1 ppm.	—	Butler 1965
Atrazine	Cockle (*Cardium edule*; Mollusc)	—	—	LC_{50} >100 ppm	—	Portmann and Wilson 1971 in Ghassemi et al. 1981
Atrazine	Freshwater benthic (bottom) organisms, primarily insects and insect larvae	Surface absorption	—	Most species reduced by at least 50% after application of atrazine at 0.5 to 2.0 ppm.	—	Walker 1962 in Pimentel 1971
Atrazine	Soil organisms	Surface absorption	—	In plots treated at 2 to 4 acres, wireworms, earthworms and springtails decreased in numbers, numbers of mites and millipedes did not change.	Faunal changes may have been due to changes vegetation rather than to direct effects of atrazine.	Fox 1964 in Pimentel 1971
Atrazine	Soil animals	Surface absorption	—	Did not cause significant reduction in number when applied at normally recommended doses.	—	Edwards 1964 in Pimentel 1971
Atrazine	Soil amoeba (22 strains)	Atrazine incorporated into culture medium	—	Atrazine at 40 and 70 ppm retarded growth of only 2 of the 22 strains tested.	—	Pons and Pussard 1980

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Soil microorganism	Atrazine incorporated into soil cultures.	56 days	Atrazine incorporated at rate equivalent to 5 tons a.i./acre (11,227 kg/ha) in laboratory resulted in 22% inhibition of CO_2 production compared to controls. Formulated wettable powder incorporated at the same rate resulted in 43% inhibition of CO_2 production. Bacterial growth was inhibited by both pure atrazine and its formulation; streptomyces growth was enhanced by pure atrazine and inhibited by its formulation; and fungis growth was unaffected by pure atrazine and inhibited by its formulation.	—	Stojanovic et al. 1972
Atrazine	Marine fungi: Ceriosporopsis halima; Leptosphaeria oraemaris; Lulworthia sp.; Dendryphiella salina; Monodictys pelagica; Periconia prolifica; Trichocladium achrasporum; and Zalerion maritimum	Atrazine incorporated into culture medium of artificial seawater.	Various, up to 24 days	Eight to 18% reduction in atrazine at 30 ppm and 9 to 68% reduction at 500 ppm.	Leptosphaeria grew with 500 ppm a.i. as sole source of either carbon or nitrogen (but not both). Periconia grew with atrazine as sole nitrogen source better than without.	Schocken et. al. 1982
Atrazine	Soil bacterium: Nocardia sp.	Atrazine incorporated into culture medium.	Not given	Nocardia exposed to 30 ppm atrazine was able to utilize the herbicide as a sole source of both carbon and nitrogen.	A metabolite produced in presence of glucose without phytotoxic activity.	Giardina et al. 1980

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Soil fungi: 29 species	Atrazine incorporated into culture medium.	Various, 1 to 14 days.	Fungi exposed to 4.4, 8.8, 17.5, 35, 70, and 140 ppm atrazine. No lethal effect observed, most species retarded in growth with percent retardation varying with atrazine concentration. One species only slightly affected, 5 species stimulated at low doses and retarded at high doses, and 1 species stimulated at higher doses up to 70 ppm.	—	Richardson 1970
Atrazine	Soil algae: Chlamydomonas reinhardi	Atrazine incorporated into culture medium.	up to 2 weeks	Autotrophic (light exposed) growth completely inhibited at at 0.5 ppm atrazine but heterotraphic (dark exposed) growth was not affected.	—	
	Chlamydomonas eugametos	Atrazine incorporated into culture medium.	up to 2 weeks	Autotrophic growth was only slightly affected at 5.0 ppm atrazine.	—	Leoppky and Tweedy 1969
	Chlorella vulgaris	Atrazine incorporated into culture medium.	up to 2 weeks	Slight toxic effect at 5.0 ppm atrazine.	—	
	Chlorella pyrenoidosa	Atrazine incorporated into culture medium.	up to 2 weeks	Marked toxic effect.	—	
Atrazine	Marine phytoplankton (Nannochloris occulata)	Atrazine incorporated into culture medium.	Not specified. Kept until a constant growth rate observed for 3 days.	At 100 ppb atrazine, percent inhibition in culture growth ranged from 46.2% at low temperature (59°F) and light to 54.0% at high temperature (77°F) and light.	—	Karlander et al. 1983

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Blue-green algae (Cyanobacteria)	Atrazine incorporated into culture medium.	—	0.01 to 5 ppm atrazine inhibited growth of all 4 algae tested.	—	Hutber et al. 1979
Atrazine	Soil amoeba (Acanthamoeba castellanii)	Atrazine incorporated into culture medium.	5 days	Growth of amoeba cultures greatly reduced at 0.1 ppm atrazine; at 1 ppm and at 10 ppm, growth arrested.	—	Prescott and Olson 1972
Atrazine (99%)	Planktonic algae	Atrazine incorporated into culture medium.	2 weeks	At a concentration of 1 ppm, none of the 12 algae isolates capable of growth in atrazine were able to remove atrazine from growth medium.	—	Bulter et al. 1975a
Atrazine (99%)	Planktonic algae (36 isolates)	Atrazine incorporated into culture medium.	2 weeks	Cultures grown at 0.001, 0.01, 0.1, 1.0, 5.0, and 10.0 ppm atrazine. Algal growth retarded at 0.01 ppm, concentrations of 1 ppm and greater were highly toxic to algae.	—	Bulter et al. 1975a
Atrazine	Soil microorganisms (Bacteria, Fungi, and Actinomycetes)	Atrazine incorporated into potted soil.	5 to 15 days	Atrazine applied at a rate of 0.89 lb a.i./acre (1 kg/ha) resulted in a decrease in soil microflora populations and CO_2 evolution measured 5 days after application. After 15 days microflora populations and CO_2 evolution approached control levels.	—	Mukhopadhyay 1980

[a]95 percent confidence limits in parentheses.
[b]Data not available or not applicable.

Appendix B

Summary of Atrazine Toxicity Data for Fish and Amphibians

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine (Technical)	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 4.5 ppm (3.5 to 5.7 ppm)[a]	Water temperature 57.2 ± 3.6°F (14 ± 2°C). Average pH = 7.2 and 10 ppm oxygen.	Bathe et al. 1976
	Crucian carp			LC_{50} 100 ppm		
Atrazine (Technical)	Rainbow trout	Intraperitoneal injection	7 days observation	LD_{50} = 1,439 mg/kg (974 to 2,124)	Water temperature 57.2 ± 3.6°F (14 ± 2°C). Average pH = 7.2 and 10 ppm oxygen.	
	Crucian carp			LD_{50} = 2,895 mg/kg (2,402 to 3,488)		
Atrazine	Bluegill	Surface absorption, flow-through bioassay	96 hours	LC_{50} = >8.0 ppm	Water temperature 66.2 ± 1.8°F (19 ± 1°C), 6.5 gram fish. Fish appeared darkened and stressed at 1.4 ppm concentration.	Macek et al. 1976
Atrazine	Brook trout	Surface absorption, flow-through bioassay	96 hours	LC_{50} = 6.3 ppm (4.1 to 9.7 ppm)	Water temperature 55.4 ± 1.8°F (13 ± 1°C), 55 gram fish. Fish appeared darkened and stressed at 1.4 ppm concentration.	
Atrazine	Fathead minnow	Surface absorption flow-through bioassay	96 hours	LC_{50} = 15 ppm (11 to 20 ppm); NOEL 8.0 ppm	Water temperature 66.2 ± 1.8°F (19 ± 1°C), 1.8 gram fish. Fish appeared darkened and stressed at 1.4 ppm concentration.	Macek et al. 1976
Atrazine	Bluegill	Surface absorption, flow-through bioassay	28 days	Fish exposed to 0.5 ppm became lethargic, fed poorly and partially lost equilibrium.	15 gram fish. Toxic effects were dose-related.	
Atrazine	Fathead minnow (fry)	Surface absorption, static bioassay	96 hours	3 to 5 day old fry exposed to 0.52 ppm exhibited 25% mortality.	Mortality was dose-related.	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Brook trout	Surface absorption	up to 44 weeks	Fish exposed to concentrations up to 0.72 ppm not affected in terms of parental survival, egg production, and hatchability. Growth and survival of fry were reduced with 90-day exposure to 0.72, 0.45 and 0.24 ppm atrazine.	--[b]	Macek et al. 1976
Gesaprim (80% wettable powder)	Trout	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} = 30 ppm LC_{50} = 17 ppm	Water temperature 57.2 ± 3.6°F (14 ± 2°C)	Bathe et al. 1975 (96-hour LC_{50} for Trout from Litchfield and Wilcoxon 1949 in Bathe et al. 1975)
	Crucian Carp	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} 100 ppm LC_{50} 100 ppm	Water temperature 57.2 ± 3.6°F (14 ± 2°C)	
	Catfish	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} 37 ppm LC_{50} = 35 ppm	Water temperature 68 ± 3.6°F (20 ± 2°C)	
	Perch	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} = 80 ppm LC_{50} = 50 ppm	Water temperature 57.2 ± 3.6°F (20 ± 2°C)	
Atrazine (wettable powder)	Bluegill	Surface absorption	96 hours	LC_{50} approximately 6 ppm	--	Walker 1964 in Lorz et al. 1979
AAtrex®	Coho Salmon	Surface absorption	144 hours	Gradual concentration-dependent mortality in freshwater with 0 mortality at 5 ppm, and 5% mortality at 8 ppm, and 25% mortality at 15 ppm.	When survivors were transferred to sea water for 244 hours, those that had been exposed to 5 ppm suffered 6% mortality, those exposed to 15 ppm suffered 25% mortality. Mortality occurred during the first 24 hours and was due to poor overall condition of the fish rather than direct osmoregulatory effects.	Lorz et al. 1979

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Bluegill	Surface absorption	up to 18 months	Fish exposed to concentrations up to 0.095 ppm were not affected in terms of survival growth, hatching, and growth of fry.	--	Macek et al. 1976
Atrazine	Fathead minnows	Surface absorption	up to 43 weeks	Fish exposed to concentrations up to 0.213 ppm were not affected in terms of survival, growth and spawning.	--	
Atrazine	Rainbow trout	Surface absorption	48 hours	LC_{50} = 12.6 ppm	--	U.S. Department of the Interior 1968 in Pimentel 1971
Atrazine	Harlequin fish	Surface absorption	24 hours	LC_{50} = 0.55 ppm	--	Alabaster 1969 in Pimentel 1971
Atrazine	Spot	Surface absorption	48 hours	No effect when exposed to 1.0 ppm.	--	Butler 1965
Atrazine	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 15 ppm	--	Klaasen and Kadoum 1979 in Spehar et al. 1981
Atrazine (granular)	Bluegill	Surface absorption	12 days	No mortality observed at 10 ppm.	Fish 1 to 3 inches in length.	Hiltibran 1967
Atrazine (wettable powder)	Bluegill	Surface absorption	12 days	No mortality observed at 5 ppm.	Fish 1 to 3 inches in length.	
Atrazine (granular)	Bluegill (fry)	Surface absorption	8 days	No mortality observed at 10 ppm.	--	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine (granular)	Green sunfish (fry)	Surface absorption	8 days	No mortality observed at 11 ppm.	--	Hiltibran 1967
Atrazine (wettable powder	Lake chub-sucker (fry)	Surface absorption	8 days	No mortality observed 10 ppm.	--	
Atrazine (wettable powder)	Smallmouth bass (fry)	Surface absorption	3 days	Fish died within 3 days when exposed to 10 ppm.	--	
Atrazine	Trout	Surface absorption	48 hours 96 hours	LC_{50} = 10 ppm LC_{50} = 8.8 ppm	Water temperature 57.2 ± 3.6°F (14 ± 2°C)	Bathe et al. 1975 (96-hours LC_{50}'s for all but the Orfe are from Litchfield and Wilcoxon 1949 in Bathe et al. 1975)
Atrazine	Crucian carp	Surface absorption	48 hours 96 hours	LC_{50} = 100 ppm LC_{50} = 76 ppm	Water temperature 57.2 ± 3.6°F (14 ± 2°C)	
Atrazine	Catfish	Surface absorption	48 hours 96 hours	LC_{50} = 8 ppm LC_{50} = 7.6 ppm	Water temperature 68 ± 3.6°F (20 ± 2°C)	
Atrazine	Perch	Surface absorption	48 hours 96 hours	LC_{50} >21 ppm LC_{50} = 16 ppm	Water temperature 57.2 ± 3.6°F (14 ± 2°C)	Bathe et al. 1975 (96-hour LC_{50}'s for all but the Orfe are from Litchfield and Wilcoxon 1949 in Bathe et al. 1975)
Atrazine	Guppy	Surface absorption	48 hours 96 hours	LC_{50} 10 ppm LC_{50} = 4.3 ppm	Water temperature 68 ± 3.6°F (20 ± 2°C)	
Atrazine	Orfe	Surface absorption	48 hours 96 hours	LC_{50} = 70 ppm LC_{50} = 44 ppm	Water temperature 57.2 ± 3.6°F (14 ± 2°C)	
Atrazine	Channel catfish	Surface absorption, flow-through bioassay	From spawning through 96 hours post hatching	LC_{50} = 0.22 ppm (0.15 to 0.32 ppm)	At exposures to 0.06, 0.43, 4.83 and 46.7 ppm atrazine, 4, 13, 69 and 100% respectively, of hatched fish displayed teratogenic effects.	Birge et al. 1979 and 1983

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Rainbow trout	Surface absorption, flow-through bioassay	From spawning through 96 hours post hatching	LC_{50} = 0.87 ppm (0.63 to 1.15 ppm)	At exposures to 0.05, 0.54, and 5.02 ppm atrazine, 3, 6 and 62% respectively, of hatched fish displayed teratogenic effects. No fish hatched from eggs exposed to 50.9 ppm atrazine.	Birge et al. 1979 1983
AAtrex® 80W	Rainbow trout	Surface absorption	96 hours	LC_{50} = 4.5 ppm	—	Ciba-Geigy Chemical Corporation data in Weed Science Society of America 1983
	Bluegill	Surface absorption	96 hours	LC_{50} > 24 ppm	—	
Atrazine	Goldfish	Surface absorption	96 hours	LC_{50} = 60 ppm	—	Geigy Agricultural Chemical Company product literature reported in U.S. Department of Agriculture 1978
Atrazine	Bullfrog	Surface absorption, flow-through bioassay	From spawning through 96 hours post hatching.	LC_{50} = 0.41 ppm (0.27 to 0.59 ppm)	At exposures to 0.4, 6.33, 14.8, 26.4 and 45.8 ppm atrazine, 3, 7, 22, 47 and 100%, respectively, of hatched toads displayed teratogenic effects.	Birge et al. 1980 and 1983
Atrazine	American toad	Surface absorption, flow-through bioassay	From spawning through 96 hours post hatching	LC_{50} > 48 ppm	At exposures to 10.8, 24.8 and 48.2 ppm atrazine, 3, 6 and 17% respectively, of hatched toads displayed teratogenic effects.	

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine	Bullfrog	Surface absorption, flow-through bioassay	From spawning to hatching.	LC_{50} = 11.55 ppm (9.8 to 13.26 ppm)	--	Birge et al. 1980
Atrazine	Leopard frog	Surface absorption, flow-through bioassay	From spawning to hatching.	LC_{50} = 22.89 ppm (17.18 to 30.01 ppm)	--	Birge et al. 1980
			From spawning through 96 hours post hatching.	LC_{50} = 7.68 ppm (4.84 to 11.90 ppm)	--	Birge et al. 1980
Atrazine	Pickerel frog	Surface absorption, flow-through bioassay	From spawning to hatching.	LC_{50} = 20.20 ppm (17.77 to 22.96 ppm)	--	Birge et al. 1980
			From spawning through 96 hours post hatching.	LC_{50} = 17.96 ppm (15.86 to 20.11 ppm)	--	Birge et al. 1980
Atrazine	Leopard frog	Surface absorption, static bioassay	96 hours	Lethal threshold = 8.5 ppm	Lethal threshold is the concentration at which one more individual died in the exposed group than in the control group.	Hovey 1975 in Hine et al. 1981
Atrazine	Leopard frog (Tadpoles)	Surface absorption	54 days	Tadpoles exposed to 0.31 to 12 ppm atrazine without significant mortality for first 27 days. After 27 days, significant increase in mortality was observed. Concentrations of 0.31 ppm significantly retarded growth.	--	Data cited in Hine et al. 1981

[a]95 percent confidence limits in parentheses.
[b]Data not available or not applicable.

Appendix C

Summary of Atrazine Toxicity for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Atrazine (99% a.i.)	Bobwhite	Ingestion	8 days (5 days in diet, 3 days observation)	LC_{50} > 5,000 ppm in feed.	No mortality to 5,000 ppm. Nine-day old birds.	Hill et al. 1975
Atrazine (99% a.i.)	Japanese Quail	Ingestion	8 days (5 days in diet, 3 days observation)	LC_{50} > 5,000 ppm in feed	No mortality to 2,500 ppm, 7% mortality at 5,000 ppm. Seven-day old birds.	
Atrazine (99% a.i.)	Ring-necked pheasant	Ingestion	8 days (5 days in diet, 3 days observation)	LC_{50} > 5,000 ppm in feed	No mortality to 5,000 ppm. Ten-day old birds.	
Atrazine (99% a.i.)	Mallard	Ingestion	8 days (5 days in diet, 3 days observation)	LC_{50} > 5,000 ppm in feed	No mortality to 2,500 ppm; 30% mortality at 5,000 ppm. Ten-day old birds.	
Atrazine (80% wettable powder)	Mallard	Ingestion	—[a]	LD_{50} > 2,000 mg/kg	Six month old males. Acute toxic symptoms were weakness, tremors, ataxia, and weight loss and lasted from 1 hour to 11 days post-treatment.	Tucker and Crabtree 1970
Atrazine (80% wettable powder)	Chickens	Ingestion by capsule	10 daily doses	Chickens treated with 10 daily doses of 0, 25, 50, 100, and 250 mg/kg had weight changes of + 45%, + 61%, + 24%, + 20%, and - 2%, respectively.	--	Palmer and Radeleff 1969
AAtrex® 80W	Chicken (female single-comb white leghorn)	Ingestion	14 days (7 days in diet, 7 days observation)	100 ppm in diet did not result in toxic symptoms, visible adverse physiological effects, or changes in egg production or egg weight.	--	Foster and Khan 1976

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
AAtrex® 80W	Bobwhite	Ingestion	8 days	LC_{50} = 5,760 ppm in feed	—	Ciba-Geigy Chemical Corporation data in Weed Science Society of America 1983
	Mallard	Ingestion	8 days	LC_{50} = 19,650 ppm in feed		
AAtrex® 80W (80% a.i.)	Pheasants (female)	Ingestion by capsule	15 weekly doses	Hen pheasants fed weekly oral doses of 0, 100, 200, and 400 mg atrazine were not affected in terms of weight gain, number of eggs laid, eggshell thickness, visual cliff performance of offspring, and survival and weight gain of offspring. Eggs of hens treated at 200 and 400 mg were lighter than controls, chicks of treated with 100 mg were more readily hand-captured than controls or chicks from hens treated with 200 or 400 mg, and there was an increase in fertility and hatchability at low doses.	Weight of hens not given.	Melius 1975
Atrazine	Bobwhite	Ingestion	8 days (5 days in diet, 3 days observation)	LC_{50} = 700 to 800 ppm	Several of the authors of the primary report have reported a Bobwhite LC_{50} of >5,000 ppm with the same protocol (Hill et al. 1975).	Heath et al. 1972 from U.S. Environmental Protection Agency registration data reported by Ghassemi et al. 1981 and cited in U.S. Department of Energy 1983
Atrazine (HCl in 90%	Chicken eggs	Injection	—	Atrazine injected into hens eggs to give 10, 100, 200, 300, and 400 ppm in the egg resulted in reduced hatching (73% of control) only at 400 ppm.	No teratogenic effects were observed.	Dunachie and Fletcher 1970

[a]Data not available or not applicable.

Appendix D

Summary of Toxicity Data for Atrazine

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Atrazine	Rat	Single dose	LD_{50} was 3,000 mg/kg	--[a]	Bashmurin 1974 in Hayes 1982
	Rat	Single dose	LD_{50} was 1,400 mg/kg	--	Gzhegotskiy et al. 1977 in Hayes 1982
Atrazine Technical, (AAtrex®)	Rat	Single dose	LD_{50} was 3,080 mg/kg	--	Geigy Agricultural Chemicals 1971a
Atrazine, 80W	Rat	Single dose	LD_{50} was 5,100 $\pm$ 400 mg/kg	--	Geigy Agricultural Chemicals 1971b
Atrazine	Mouse	Single dose	LD_{50} was 1,750 mg/kg	--	Bashmurin 1974 in Hayes 1982
Atrazine, Technical (AAtrex®)	Mouse	Single dose	LD_{50} was 1,750 mg/kg	--	Geigy Agricultural Chemicals 1971a
			Acute Intraperitoneal Toxicity		
Atrazine	Rat	I.P. injection	LD_{50} was 125 mg/kg	--	Gzhegotskiy et al. 1974 in Hayes 1982
			Acute Dermal Toxicity		
AAtrex® (80W)	Rat	--	LD_{50} was 9,300 $\pm$ 990 mg/kg	--	Geigy Agricultural Chemistry 1971b
			Skin Irritation		
Atrazine	Rat	--	2,800 mg/kg produced erythema but no systemic effects.	--	Gzhegotskiy et al. 1977 in Hayes 1982

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Inhalation Toxicity		
Atrazine, Technical	Rat	Rats exposed to aerosol dust containing 1.6 ppm a.i.	No evidence of toxicity.	--	Geigy Agricultural Chemicals 1971a
			Eye Irritation		
Atrazine	Rat	Not specified	"Irritating"	--	Gzhegotskiy et al. 1974 in Hayes 1982
			Subchronic Oral Toxicity		
Atrazine (80W)	Cattle	Fed at 100 ppm to cows for 21 days or 30 ppm to cattle for 28 days.	No observable effects.	--	Geigy Agriculture Chemicals 1971a
Atrazine	Rats	Fed at dietary levels of 100 and 500 ppm for 6 months.	Growth retardation. No other effects.	Due in part to reduction of food intake.	Suschetet et al. 1974
Atrazine	Sheep	Fed on hay treated with 22.5 and 44.9 kg atrazine/hectare (determined to contain an average of approximately 500 and 760 ppm) for 4 weeks for a total intake of approximately of 865 and 1,200 mg/kg body weight.	No significant adverse effects observed.	Endpoints included blood chemistry, food intake body weight, histopathology.	Johnson et al. 1972
Atrazine	Calf	Fed on hay containing approximately 500 and 760 ppm for 4 weeks.	No significant adverse effects observed.	Endpoints included blood chemistry, food intake body weight, histopathology.	Johnson et al. 1972

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity		
Atrazine	Cattle, dog, horse, rat	Animals fed 25 ppm over "extended periods."	No observable ill effects.	—	Geigy Agricultural Chemicals 1971a
Atrazine	Rat	Fed in diet at 100 ppm for 2 years.	No gross or microscopic effects observed.	—	National Research Council 1977 in U.S. Department of Agriculture 1978
			Reproduction and Teratogenicity		
Atrazine	Rat	Rats were fed at dietary levels of 50, 100, 200, 300, 400, 500 and 1,000 ppm from day one throughout gestation.	No effect on the number of pups per litter or their weights at weaning was observed.	—	Peters and Cook 1973
Atrazine	Rat	Rats were injected subcutaneously with atrazine at the level of 50, 100, 200, 800, 1,000 and 2,000 mg/kg body weight on days 3, 6 and 9 of gestation.	No effect on the number of pups/litter was observed at levels up to 200 mg/kg. Levels of 800 mg/kg or higher were embryotoxic. Resorptions occurred in most of the litters. The critical day of treatment appeared to be day 6.	—	
Atrazine	Mouse	Mothers dosed with 46.4 mg/kg from days 6 through 14 of gestation.	No significant increase in fetal anomalies in three strains of mice tested.	—	Mrak 1969 in Hayes 1982
Atrazine	Sheep	Ewes treated with 15 mg/kg/day throughout pregnancy.	No adverse effects. A dosage of 30 mg/kg/day killed pregnant and unpregnant ewes in 36 to 60 days.	—	Binns and Johnson 1970 in Hayes 1982

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity		
Fogard S® (25% atrazine and 37.5% simazine)	Mouse	One group of animals given 13 subcutaneous injections at 3 day intervals for a total of 0.0065 mg active ingredient. Second group was treated similarly, but with interperitoneal injections for a total of 0.0065 mg active ingredient.	Increased incidence of malignant lymphomas was observed.	—	Donna et al. 1981
Atrazine	Mouse	Mice treated with 21.5 mg/kg by stomach tube from days 7 through 28 of age followed by 82 ppm in the diet for about 18 months.	No gross or microscopic evidence of any significant increase in tumors.	—	Innes et al. 1969

[a]Data not available or not applicable.

Appendix E

Summary of Mutagenicity Data for Atrazine

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Atrazine	Salmonella typhimurium	Eight histidine requiring mutants were treated with a "small crystal" per plate.	Nonmutagenic, no induction of revertant colonies.	--[a]	Andersen et al. 1972
Atrazine	S. typhimurium	In the Salmonella/ microsome assay, Strains TA1535, TA1538, TA98 and TA100 were treated with atrazine at doses up to 5 mg/plate or which were toxic.	Nonmutagenic, no increase in reversion to histidine prototrophy (i.e. wild type).	Test done in presence and of rat liver S-9 metabolic activation.	Simmon et al. 1977
Atrazine	S. typhimurium	Tested for reverse mutation at the histidine marker in a host-mediated assay. Tested in the presence of animal and plant microsomes for metabolic activation. Animals treated with 1,000 mg/kg body weight.	Nonmutagenic	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	S. typhimurium	Tested in a forward mutation assay for 8-azaguanine instance. Tested at 4.62×10^{-3} to 2.3×10^{-2} in mM per plate.	Nonmutagenic	Summary report. No details presented.	
Atrazine	S. typhimurium	Strains his G46, TA1530, TA1531, TA1532, and TA1534 used in a reverse mutation assay. Doses and conditions of test unspecified.	Nonmutagenic for reversion to histidine independence.	Summary report. Probably tested in the absence of metabolic activation.	Seiler 1973
Alachlor (34% a.i.)	S. typhimurium	Strains TA98, TA100, TA1537, TA1538 and TA1978 used in a reverse mutation assay. Tested at 0.001 to 5% in the absence of metabolic activation.	Positive mutagenicity with strains TA981, TA 1535, TA1537, and TA 1978.	Summary report. No numerical data presented.	Njagi and Gopalan 1980

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Atrazine® (AAtrex 80W)	Saccharomyces cerevisiae Strain D4	Maize seedlings were exposed to 10-25 ppm atrazine. Extracts (1S fraction) from the plants were applied to the yeast culture in a mitotic gene conversion assay.	Increased frequency of mitotic gene conversion.	Atrazine alone was not mutagenic to S. cerevisiae indicating that the compound is converted to a mutagenic form by the plant since plant extracts are mutagenic.	Plewa and Gentile 1976
Atrazine	S. cerevisiae	Tested for gene conversion conditions	Nonmutagenic in the presence of mammalion metabolism.	Summary report. No details presented.	deBertoldi et al. 1978
Atrazine	S. cerevisiae	Mitotic gene conversion assay. Marker at trp 5-12/27 locus. Tested at 2.5 to 10 mM in the presence of potato microsomes.	Nonmutagenic	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	S. cerevisiae	Strain D4-RDII was used in a direct or host mediated mitotic gene conversion assay. Doses or conditions were not specified.	Nonmutagenic	Summary report. No details presented	Marquardt 1980
Atrazine	S. cerevisiae	Strain D4 used in a mitotic gene conversion assay. Tested at 1,000 and 4,000 ppm in the presence of mouse liver microsomes.	Nonmutagenic	—	deBertoldi et al. 1980
Atrazine	Escherichia coli	Tested in a host-mediated mutagenicity test in which test compound is given orally to mice, bacteria are injected intravenously then recovered several days later and screened for forward mutations in antibiotic-resistance markers. Also tested in an in vitro bacterial mutagenicity test with liver homogenate metabolic activation. Conditions unspecified.	Mutagenic when atrazine was given orally to mice in the host-mediated test, but negative in the in vitro test.	Summary report. No details presented.	Solt and Neal 1980

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Atrazine	E. coli	Tested in a forward mutation host-mediated assay for resistance to ampicillin. Tested at 0.5 to 2.0 mM. Animals dosed orally with 10 mg/kg body weight for possible host-mediated activation.	Nonmutagenic	Summary report. No details presented.	deBertoldi et al. 1978
Atrazine	Aspergillus nidulans	Tested for gene conversion. Conditions unspecified.	Nonmutagenic	Summary report. No details presented.	
Atrazine	A. nidulans	Forward mutation assay for resistance to 8-azaguanine. Tested inthe presence of plant microsomes for metabolic activation. Doses unspecified.	Mutagenic	Summary report. No details presented.	
Atrazine	A. nidulans	Tested for mitotic crossing over. Marker at the fpa A1 locus. Tested in the presence of potato microsomes at unspecified doses.	Mutagenic	Summary report. No details presented.	deBertoldi et al. 1978
Atrazine	A. nidulans	Diploid strain used in a mitotic gene conversion assay. Tested at 500 to 8,000 ppm.	Nonmutagenic	--	
Atrazine	A. nidulans	Tested for induction of forward gene mutations (8-azaguanine resistance) and mitotic crossing over. Tested at 1 to 5 mg/plate with metabolites from treated Nicotiana plants.	Positive for 8-azaguanine resistance and mitotic crossing over only after treatment with plant metabolites.	--	Carere and Morpugo 1981
Atrazine	Schizosaccharomyces pombe	Tested for forward mutations. Conditions unspecified.	Mutagenic; increase in forward mutations.	Summary report. No details presented.	
Atrazine	S. pombe	Forward mutation host-mediated assay. Markers at 5 loci. Animals treated orally with 1,000 mg/kg body weight.	Mutagenic when tested in vitro at 3.0 mM or greater in the presence of either potato microsomes or animal S-9 fraction.	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	Streptomyces coelicolor	Forward mutation assay for resistance to streptomycin. Doses unspecified.	Mutagenic in the presence of potato microsomes.	Summary report. No details presented.	

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Atrazine	Bacteriophage T_4	Cultures treated with 20 μg/plate.	No induction of mutants.	--	Andersen et al. 1972
Atrazine	rII mutants of bacteriophage T_4	Cultures treated with 1,000 μg/plate.	No reversion of mutants to to wild type bacteriophage.	--	Andersen et al. 1972
AAtrex®80W (80% atrazine)	Drosophila melanogaster	Assays for dominant lethal mutations, sex-linked recessive lethal mutations, chromosomal breakage, nondisjunction and loss were done. Adults or larvae were appropriately injected or fed atrazine at 0.01%.	A significant increase in rate of dominant lethals was observed. X-Linked recessive lethals occurred when atrazine was injected but not when fed. There was no significant increase in partial loss of the Y chromosome and no sex chromosome disjunction was observed.	Authors ascribed increase in dominant lethals to a reduction in sperm transferred from treated males or to toxicity.	Murnik and Nash 1977
Atrazine	D. melanogaster	Tested for recessive lethals at 5 and 10 mM in vivo.	Nonmutagenic	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	V79 hamster cells	Forward mutation assay for resistance to 6-thioguanine.	Mutagenic when tested at 3.0 mM or greater in the presence of potato microsomes.	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	Chinese hamster cells	Tested for induction of chromosome aberrations at concentrations of 12.5 to 100 mM, with and without metabolite activation. Conditions unspecified.	No cytogenetic damage observed.	Summary report. No details presented.	Sobels 1980
Atrazine	CHO hamster cells	Tested for chromosome breakage at 1.25 to 10 mM.	Negative		
Atrazine	CHO hamster cells	Tested for sister chromatid exchanged at doses of 1.25 to 10 mM.	Negative for sister chromatid exchanges.	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	EUE human cells	Tested for unscheduled DNA synthesis. Conditions unspecified.	Positive for unscheduled DNA synthesis when tested at doses of 3.0 mM or greater in the presence of potato microsomes.		

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Atrazine	Mouse bone marrow cells	Tested for chromosome aberrations. Animals treated with 2,000 mg/kg body weight.	Increased induction of chromosome breaks (4% vs 0.7% in controls).	—	Ehling 1980
Atrazine	Mouse bone marrow cells	Tested for chromosome aberrations. Animals treated with single dose of 1,000 or 2,000 mg/kg body weight.	Increased induction of chromosome aberrations.	Summary report. No details presented.	Loprieno et al. 1980
Atrazine	Mouse	Tested for dominant lethals. Males dosed orally with 1,500 mg/kg body weight.	Negative	Summary report. No details presented.	Loprieno and Adler 1980
Atrazine	Mouse	Tested for dominant lethal effects. Male mice dosed orally with 1,500 and 2,000 mg/kg body weight.	Induction of dominant lethal mutations.	—	Ehling 1980
Atrazine	Barley	Seeds or seedlings were soaked (12 hrs) or sprayed with solution of herbicide at 1,000 ppm.	Induced chromosomal aberrations in root tip cells at frequencies greater than that induced in controls by 5,500 R of x-rays.	—	Wuu and Grant 1966a,b
Atrazine	Barley	Seeds soaked in or sprayed with a solution containing 1,000 ppm.	Induced chromosomal aberrations in meiotic cells of plants.	—	Wuu and Grant 1967
Atrazine	Maize plants	Plants were grown in the presence of 35.3 mg atrazine. At the anthesis stage tassels were removed and pollen grains scored for reversion frequency at the waxy locus to determine effect on plant germ cells.	Increased frequency of reversion at the waxy locus.	—	Plewa and Gentile 1976
Atrazine	Maize seedling roots	Maize seedlings exposed to Atrazine at 10 and 20 ppm.	Increased frequency of sister chromatid exchanges.	—	Chou and Weber, 1981

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Atrazine

Formulation	Organism	Dose	Comments	References
Atrazine (AAtrex® 80W)	Chicken (Single Comb White Leghorn hens)	100 ppm in feed for 7 days followed by 7 days without atrazine in feed before sacrifice.	A high concentration (38.8 ppm) of unchanged atrazine found in abdominal fat, 0.04 ppm found in leg muscle, and no atrazine detected in other tissues. Three metabolites found in various tissues-up to 2.33 ppm deethylatrazine in gizard, up to 16.18 hydroxyatrazine in liver and 4.28 ppm in kidney, and 15.5 ppm deethylhydroxatrazine in liver and 2.29 ppm in kidney.	Khan and Foster 1976
Atrazine (99.2% a.i.)	Whitefish	Fish immersed in water with 210 to 284 ppb atrazine for up to 30 days without feeding or immersed in water with 54 to 389 ppm atrazine for up to 30 days while fed non-contaminated food.	Initial (up to 2 or 3 days) concentration factors of 3 to 6 were observed followed by a leveling off to a concentration factor of 2 to about 3. In starved fish, the highest concentration (factor 20) was in gall bladder followed by liver, brain and gut (2 to 15). Small residues found in skeletal muscle. In fish fed non-contaminated food, similar concentration patterns occurred with high initial residues in liver, brain, gills and muscle with gill, muscle and liver residues decreasing over time with continued exposure.	Gunkel 1981; Gunkel and Streit 1980
Atrazine (99.2% a.i.)	Whitefish	Fish fed contaminated (0.596 ppb atrazine) food for 24 days.	Average residues in fish about 0.25 ppb. Of the total offered atrazine, 10% less accumulated per day after day 10.	Gunkel 1981

Appendix F (Continued)

Formulation	Organism	Dose	Comments	References
Atrazine (AAtrex® 80W)	Chicken (Single Comb White Leghorn hens)	100 ppm in feed for 7 days followed by 7 days without atrazine in feed.	Unchanged atrazine and 3 metabolites detected in excrement collected 24 hours after initial treatment and continued up to 4 days of uncontaminated feed. Major metabolite was deethylated atrazine with levels up to 3.52 ppm in excrement (4 days after start of atrazine diet), followed by hydroxyatrazine with levels of up to 1.13 ppm in excrement (5 days after start of atrazine diet. Maximum atrazine levels in excrement were 0.52 ppm on the 7th day of atrazine in feed.	Khan and Foster r1976
Atrazine (^{14}C)	Rat	Rats dosed by stomach tube with 0.53 mg or 5.5 mg atrazine.	Rats given 0.53 mg atrazine excreted 65.5% of the total radioactivity in urine within 72 hours (mostly, 85 to 95%, in 24 hours), 20.3% excreted in feces and 15.8% remained in body tissues with fat and muscle tissue having the least residues. Rats given the 5.5 mg dose excreted 92.9% of total radioactivity in the urine as metabolites.	Bakke et al. 1972a
Hydroxyatrazine (^{14}C)	Rat	Rats dosed by stomach tube with 0.5 mg or 5.0 mg hydroxyatrazine.	Rats given 0.5 mg hydroxyatrazine excreted 78.0% of the total radioactivity in urine within 24 hours and 5.5% was excreted in the feces. A total of 83.5% of the activity was accounted for. Rats dosed with 5.0 mg of hydroxyatrazine excreted 84% of the total radioactivity in the urine as metabolites, including hydroxatrazine.	Bakke et al. 1972a

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2,4-D Summary

Major Trade Names: Numerous trade names are used.

Chemical Name: (2,4-dichlorophenoxy)acetic acid

Major Forestry Applications: Conifer release, noxious and poisonous weed control, range improvement, right-of-way maintenance, site preparation, aquatic weed control, general weed control, thinning, timber management, wildlife habitat improvement, range management, research and engineering, recreation management, firebreak management, and nursery stand improvement.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Toxicity of 2,4-D to aquatic organisms depends upon the specific formulation. Most ester formulations are toxic to highly toxic to aquatic invertebrates and fish, while salt and acid formulations are generally only slightly toxic to these organisms. In general, 2,4-D and its formulations have a low toxicity to birds.

Acute and chronic toxicity studies in mammals revealed gastroenteritis, skeletal and cardiac myotonia, and central nervous system depression following ingestion of large doses of 2,4-D. Similar clinical symptoms have been observed in human case reports. In mammals, 2,4-D is moderately toxic having acute oral LD_{50}'s in the range of 300 to 1,000 mg/kg except in dogs (LD_{50} = 100 mg/kg). Various salts and esters of 2,4-D are mild skin, eye, and respiratory tract irritants. In chronic toxicity studies in mammals, both the subcutaneous and oral routes of 2,4-D exposure caused adverse effects including renal gout, stomach ulcers, anemia, and death. Although increased tumor incidences were observed in rats (treated orally with 2,4-D) and mice (treated by subcutaneous injection with the isooctyl ester of 2,4-D), because of the small number of animals tested and possibly inadequate reporting of the data, the evaluation of the carcinogenicity of 2,4-D is inconclusive. In reproduction and teratogenesis studies, decreases in birth weight, litter size, and fertility were observed; however, these adverse effects were caused by high doses that caused maternal toxicity. Epidemiology studies

have suggested an increased cancer risk in humans exposed to phenoxy acids and chlorophenols. Because of the concurrent exposure in these studies to 2,4-D and other chemicals, possibly including the contaminant 2,3,7,8-tetrachlorodibenzo-para-dioxin, the carcinogenic potential of 2,4-D alone cannot be evaluated. Generally, 2,4-D has been found to be nonmutagenic in most of the microbial systems investigated.

Environmental Fate

Uptake of 2,4-D by plants occurs through leaves, stems, and roots. It is translocated within the plant to regions of growth where it interfers with normal growth processes. Plants metabolize 2,4-D readily by a variety of pathways to various degradation products. Animals do not bioaccumulate 2,4-D to any great extent. The 2,4-D that is absorbed is usually eliminated very rapidly in unmetabolized form. Esters of 2,4-D, however, are usually hydrolyzed to the free acid form in animals prior to excretion.

In soils, 2,4-D generally has a short (one month or less) persistence. It tends to be mobile and is primarily degraded by microbial activity. In aquatic environments, 2,4-D is readily degraded by microbial activity, which, along with dilution, is the major means for its loss of activity in aquatic systems. Some photodegradation takes place in surface waters and highly volatile formulations vaporize to some extent.

Exposure and Hazard

The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels anticipated for USDA Forest Service applications.

1.0 Introduction

The herbicidal and plant growth-regulating properties of 2,4-D were first discovered as a result of secret research during World War II (Loos 1975). Since the war, 2,4-D has become the most extensively used herbicide in forestry (Norris et al. 1983). It is one of the phenoxy herbicides, which, in addition to 2,4-D, includes 2,4,5-T, 2,4-DP (Dichlorprop), 2,4,5-TP (Silvex), and MCPA in addition to others (Norris 1981). 2,4-D is also known under a variety of trade names* or other names, including Agrotect®, Amoxone, Aqua-Kleen®, Chipco Turf Hericide "D"®, Chloroxone®, Crop Rider®, Decamine®, Dedweed®, Dinoxol®, DMA®, Emulsamine BK®, Emulsamine E-3®, Envert DT®, Envert 171®, Esteron®, Estone®, Fernesta®, Fernimine®, Fernoxone®, Formula 40®, Lawn-Keep®, Lo-Vol 4D, Macondray®, Pennamine® D, Planotox®, Plantgard®, Rhodia®, Salvo®, Super D Weedone, Transamine®, Tributon®, Verton®2D, Visko-Rhap®, Weed-B-Gon®, Weedar®, Weedone®, and Weed-Rhap® (Meister Publishing Company 1981). 2,4-D is marketed alone and as a combined formulation with other herbicides, including MSMA, 2,4,5-T, and MCPP (Meister Publishing Company 1981).

The various commercial formulations of 2,4-D are generally either salt or ester formulations. Salt forms of 2,4-D are most readily absorbed through plant roots, whereas the ester forms are most readily absorbed through foliage. Both forms are readily translocated within the plant where the herbicide tends to accumulate at the meristematic areas of both shoots and roots. Although the primary mode of action of 2,4-D has yet to be determined, various studies have shown it to interfere with normal cell growth and to impair respiration, food reserves, and cell division (Weed Science Society of America 1983).

Since their introduction, 2,4-D and the other phenoxy herbicides have been studied and reviewed extensively in terms of toxicity and health effects, environmental fate, and mode of action. This background statement will not attempt to comprehensively review all that has been written about 2,4-D, but rather, will summarize what is known about its toxicity, health effects, environmental fate, and mode of action, with emphasis on studies that have been conducted over the past two decades. Reviews of 2,4-D can be found in Norris (1981), Norris et al. (1983), Ghassemi et al. (1981), Loos (1975), Mullison (1981), Minnesota Department of Health (1978), Lommen (1980), and International Agency for Research on Cancer (1977) among others.

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*Trade names are used only to provide information and do not imply product endorsement.

1.1 Normal Use Patterns

Since its introduction as a selective weed killer in the mid-1940's, 2,4-D has become one of the most widely used herbicides in the world, and the most widely used herbicide in the United States for forestry application. In 1976, approximately 240,200 lbs (108,950 kg) of 2,4-D were applied to lands administered by the USDA Forest Service as part of the National Forest System (Norris 1981). In 1975, approximately 59,500,000 lbs (27 million kg) of 2,4-D acid equivalent were used in the United States, most of which (58 percent) were applied to wheat, corn, sorghum, rice, fruit, and other grains (International Agency for Research on Cancer 1977).

Phenoxy herbicides such as 2,4-D are particularly useful, since they are toxic to a wide variety of broadleaved weeds but not to most species of the grass family, such as cereal crops and plants grown for pasture forage and ornamental lawns (Loos 1975). 2,4-D is registered for use on grasses, wheat, barley, oats, sorghum, corn, and sugarcane, as well on noncrop areas for post-emergent control of thistle, dandelion, annual mustards, ragweed, and lambs quarters. Some formulations are registered for pine release, water hyacinth control, and prevention of seed formation (Meister Publishing Company 1981). Use of 2,4-D by the USDA Forest Service includes conifer release, noxious and poisonous weed control, range improvement, right-of-way maintenance, site preparation, aquatic weed control, general weed control, thinning, timber management, wildlife habitat improvement, range management, research and engineering, recreation management, firebreak management, and nursery stand improvement (Gross 1983). Application rates ranged from 0.5 to 8.5 lb a.i. (active ingredient) per acre (0.56 to 9.5 kg/ha) for 163 USDA Forest Service projects in which 2,4-D was used in 1982. These projects used either 2,4-D amine formulations or a low-volatile ester formulation of 2,4-D. Statistics on use of 2,4-D by the USDA Forest Service are given in Table 1-1.

Recommended rates for application of 2,4-D for post-emergence control of weeds in most crops and pastures range from 0.25 to 2 lb/acre (0.28 to 2.2 kg/ha). Brush control applications usually require spray mixtures of 3 to 4 lb/100 gallons. Depending upon the specific formulation, water, diesel oil, or both are used as the carrier. Low volumes of spray mixture (3 to 10 gallons per acre) are used for aerial applications and higher volumes (up to 400 gallons per acre) are used for ground applications. Some formulations are enhanced by the use of surfactants (Weed Science Society of America 1983).

There are a variety of commercial formulations of 2,4-D available in the United States and abroad, either of 2,4-D alone or

Table 1-1

USDA Forest Service 2,4-D Use

Purpose	Active Ingredient Per Acre (lbs) Range	Active Ingredient Per Acre (lbs) Average	Acres Treated	Formulation	Application Method
		1981			
Aerial[a]					
Confier release	1.9 - 2.6	2.6	6,127	Liquid	Helicopter
Noxious weeds	2.0	2.0	163	Liquid	Helicopter
Range improvement	1.5 - 1.9	1.6	22,527	Liquid	Helicopter, Fixed-wing
Right-of-way	0.8 - 7.8	3.0	1,232	Liquid	Helicopter
Site preparation	2.2 - 3.4	2.9	3,784	Liquid	Helicopter
Ground[b]					
Aquatic weed control	2.0 - 18.9	12.1	19	Granule	Hand broadcast
Conifer release	0.2 - 3.4	1.4	39,376	Liquid	Injection, Broadcast, Cut Stump
General weed control	0.5 - 3.8	1.0	445	Liquid	Individual plant, Broadcast
Noxious weeds	0.5 - 3.1	1.5	9,799	Liquid	Individual plant, Broadcast
Poisonous plant control	0.7 - 2.4	0.9	235	Liquid	Individual plant
Range improvement	1.5 - 2.0	1.5	1,877	Liquid	Broadcast
Right-of-way	1.2 - 4.1	2.4	3,231	Liquid	Individual plant, Broadcast
Site preparation	0.4 - 3.3	1.3	47,477	Liquid	Injection, Individual plant, Broadcast
Thinning	0.8 - 1.8	0.9	3,493	Liquid	Cut Stump, Injection
Wildlife habitat improvement	0.6 - 1.8	1.1	5,097	Liquid	Injection, Basal spray, Individual plant

Table 1-1 (Concluded)

Purpose	Active Ingredient Per Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
	1982				
Aerial[c]					
35 Projects for Timber, Range, Noxious weeds Wildlife, Right-of-way, Firebreak	1.0 - 4.0	2.6	35,658	Low volatile ester	--
Ground[d]					
84 Projects for Research, Engineering, Timber, Range, Noxious Weeds, Wildlife, Recreation, Nursery	1.0 - 4.0	2.4		Low volatile ester	Ground Foliar
19 Projects for Range, Noxious Weeds, Wildlife, Recreation, Engineering, Nursery, Timber	0.5 - 6.0	2.5	91,152 [total]	Amine	Ground Foliar
25 Projects for Timber and Wildlife	1.0 - 8.5	3.8		Amine (undiluted)	Injection and Cut Stump

[a]Includes 353 acres right-of-way, 1,244 acres site preparation using 2,4-D/picloram 4:1 (Tordon® 101), and 1,062 acres conifer release using 2,4-D/2,4-DP (Weedone® 170).

[b]Includes the following mixes of herbicides with 2,4-D:

Dicamba - 391 Ac. conifer release
106 Ac. general weed control
647 Ac. noxious weeds
311 Ac. right-of-way
41 Ac Thinning
116 Ac wildlife habitat improvement
41 Ac. thinning
116 Ac. wildlife habitat improvement

2,4-DP (Weedone® 170) - 55 Ac. conifer release
253 Ac. right-of-way

Picloram (Tordon® 101) - 19304 Ac. conifer release
1015 Ac. noxious weeds
165 Ac. poisonous plants
339 Ac. right-of-way
55 Ac. range improvement
41349 Ac. site preparation
2982 Ac. thining
3055 Ac. wildlife habitat improvement

[c] Includes 198 Acres site preparation with 2,4-D and picloram (Tordon® 101) and 1,078 acres site preparation with 2,4-D and 2,4-DP (Weedone® 170).

[d] Includes the following mixes of herbicides with 2,4-D:

Dicamba - 2,462 Ac noxious weeds
131 Ac. range
209 Ac wildlife habitat improvement

2,4-DP - 100 Ac right-of-way

Picloram - 1,943 Ac. conifer release
1,510 Ac. noxious weeds
10 Ac. range
374 Ac. right-of-way
427 Ac. thinning
645 Ac. wildlife habitate improvement

Source: Gross 1983

in combination with other herbicides. Table 1-2 lists some of the commercial formulations of 2,4-D available in the United States. Amine salts are particularly important, although inorganic salts of sodium, potassium, and ammonium are also used. Esters, which are oil soluble, generally are more effective against plants because they are more readily absorbed. Low-molecular-weight alcohol esters have a relatively high volatility, and may therefore present a potential hazard to nearby nontarget crops. Esters of heavier molecular weight, as used by the USDA Forest Service, have a relatively low volatility and do not present such a hazard (Loos 1975).

In the United States, 2,4-D is manufactured and marketed by several chemical companies, including Diamond Shamrock, Dow, Vertac, Union Carbide, and Farmland Industries (Weed Science Society of America 1983). Combinations with other herbicides include MSMA, dicamba, picloram, and MCPP (Meister Publishing Company 1981 and Weed Science Society of America 1983).

1.2 Chemical and Physical Properties

The active ingredient of 2,4-D is (2,4-dichlorophenoxy)acetic acid. Table 1-3 illustrates the structural formula and selected properties of 2,4-D acid, the dimethylamine salt of 2,4-D, the n-oleyl-1,3-propylenediamine salt of 2,4-D (oil soluble amine salt), and the butoxyethanol ester of 2,4-D. The essential practical differences in the various forms of 2,4-D relate to their solubility, their volatility, and as a consequences of their solubility, their absorption by target plants. In general, the amine salts of 2,4-D are highly soluble in water and therefore can be used in water-based formulations. An exception to this generalization is the n-oleyl-1,3-propylenediamine salt of 2,4-D which is insoluble in water but soluble in most organic solvents. The ester forms of 2,4-D are likewise oil soluble and require the inclusion of an emulsifying agent if they are to be applied as an aqueous solution. Because of their high degree of solubility in oils and most organic solvents, the ester forms of 2,4-D generally tend to be more readily absorbed by plants and consequently have a stronger toxic effect on target species. The use of surfactants in 2,4-D formulations (acids and ester formulations) often leads to increased efficacy as a consequence of increased absorption of the herbicidal compound as the surfactants tend to dissolve surface waxes, loosen cuticular structure, and, in some cases, interact with cuticular lipids (Loos 1975).

Esters of low-molecular-weight alcohols, such as the methyl ester and the ethyl ester of 2,4-D, have relatively high vapor pressures (23×10^{-4} and 11×10^{-4} mm Hg, respectively, at 77°F

Table 1-2

Selected 2,4-D Formulations[a]

Formulation	Active Ingredients	Manufacturer[b]
Amoco 2,4-D LV Ester-M	Propylene glycol butyl ether ester of 2,4-D ..... 72.8% (44.9% acid equivalent)	Amoco
Amoco 2,4-D Weed Killer No. 3-E-M	Isopropyl esters of 2,4-D ..... 37.7% Butyl esters of 2,4-D ..... 39.7% (63.5% acid equivalent)	Amoco
Amoco 2,4-D Weed Killer No. 2-M	Dimethylamine salt of 2,4-D ..... 49.3% (40.9% acid equivalent)	Amoco
Amoco 2,4-D Weed Killer No. 5-M	Butyl ester of 2,4-D ..... 58.6% (46.76% acid equivalent)	Amoco
Aqua-Kleen 20	Butoxy ethanol ester of 2,4-D 29.0%	Amchem
Chipman 2,4-D Amine No. 4	Dimethylamine salt of 2,4-D 49.8%	Rhodia, Chipman Division[c]
Chipman 2,4-D Amine No. 6	Dimethylamine salt of 2,4-D 70.1%	Rhodia, Chipman Division[c]
Chipman 2,4-D Butyl Ester 6	n-Butyl ester of 2,4-D 80.3%	Rhodia, Chipman Division[c]
Chipman 2,4-D Butyl Ester 4E	Butyl ester of 2,4-D 59.2%	Rhodia, Chipman Division[c]
Chipman 2,4-D Butyl Ester 6E	Butyl ester of 2,4-D 79.6%	Rhodia, Chipman Division[c]
Chipman 2,4-D Butyl Ester 334E	Butyl ester of 2,4-D 49.0%	Rhodia, Chipman Division[c]
Chipman 2,4-D Gran 20	Isooctyl ester of 2,4-D 30.15%	Rhodia, Chipman Division[c]
Chipman 2,4-D Low Volatile Ester 6	Isooctyl ester of 2,4-D 95.6%	Rhodia, Chipman Division[c]

Table 1-2 (Continued)

Formulation	Active Ingredients	Manufacturer[b]
Chipman 2,4-D Low Volatile Ester 4L	Isooctyl ester of 2,4-D 94.8%	Rhodia, Chipman Division[c]
Chipman 2,4-D Low Volatile Ester 6L	Isooctyl ester of 2,4-D 94.8%	Rhodia, Chipman Division[c]
Crop Rider 20% Aqua Granular	Isooctyl ester of 2,4-D 30.17%	Diamond Shamrock
Crop Rider 2.67D	Butyl ester of 2,4-D 40.0%	Diamond Shamrock
Crop Rider 3.34D	Isopropyl ester of 2,4-D 46%	Diamond Shamrock
Crop Rider 6D-OS Weed Killer	Butyl ester of 2,4-D 78%	Diamond Shamrock
Diamond Shamrock Amine 4D	Dimethylamine salt of 2,4-D 49%	Diamond Shamrock
Diamond Shamrock Amine 6D	Dimethylamine salt of 2,4-D 70%	Diamond Shamrock
Diamond Shamrock Butyl 4D	Butyl ester of 2,4-D 56.5%	Diamond Shamrock
Diamond Shamrock Butyl 6D Weed Killer	Butyl ester of 2,4-D 78%	Diamond Shamrock
Diamond Shamrock LO-Vol 4D	Isooctyl ester of 2,4-D 69%	Diamond Shamrock
Diamond Shamrock LO-Vol 6D	Isooctyl ester of 2,4-D 92.8%	Diamond Shamrock
Emulsamine E-3	Dodecyl amine salts of 2,4-D ..... 50.7% Tetradecyl amine salts of 2,4-D 12.7%	Amchem
Esteron 6E Herbicide	Isooctyl ester of 2,4-D ..... 94.4% (63.2% acid equivalent)	Vertac
Esteron 76BE Herbicide	Butyl esters of 2,4-D ..... 79.2% (63.2% acid equivalent) in petroleum solvent	Vertac
Esteron 44 Improved Weed Killer	Butyl esters of 2,4-D ..... 51.0% in emulsifiers and petroleum solvent	Vertac
Esteron® 99 Weed Killer	Propylene glycol butyl ether esters of 2,4-D ..... 41.0% in petroleum solvent	Vertac

Table 1-2 (Continued)

Formulation	Active Ingredients	Manufacture[b]
Esteron® 99 Weed Killer Concentrate	Propylene glycol butyl ether esters of 2,4-D ..... 72.8% in petroleum solvent	Vertac
Pennamine D	Heptylamine salt of 2,4-D ..... 4 lb/gal. (liquid concentrate)	Pennwalt
Weedeth	Dimethylamine salt of 2,4-D 49%	Miller
Weedez Wonder Bar	Triethylamine salt of 2,4-D 18.0%	Rhodia, Chipman Division[c]
Weedone 638	2,4-Dichorophenoxyacetic acid 31.0%	Amchem
Weedone Aero-Concentrate 96	Butyl ester of 2,4-D 75.2%	Amchem
Weedone Aero-Concentrate E	Butyl ester of 2,4-D 76.0%	Amchem
Weedone Concentrate 48	Ethyl ester of 2,4-D 38.6%	Amchem
Weedone LV4	Butoxy ethanol ester of 2,4-D 62.5%	Amchem
Weedone LV6	Butoxy ethanol ester of 2,4-D 88.1%	Amchem
Weed-Rhap A-4D	Dimethylamine salt of 2,4-D ..... 49.5% (41.1% acid equivalent)	Transvaal
Weed-Rhap B-4D	Butyl ester of 2,4-D ..... 58.8% (46.9% acid equivalent)	Transvaal
Weed-Rhap B-6D	Butyl ester of 2,4-D ..... 79.1% (63.1% acid equivalent)	Transvaal
Weed-Rhap LV-4D	2-Ethylhexyl ester of 2,4-D ..... 69.9% (46.3% acid equivalent)	Transvaal
Salvo Weed Killer	2-Ethylhexyl ester of 2,4-D 86.7%	Olin
Transamine OA-3D	N,N-Dimethyloleyl-linoleyl amine salt of 2,4-D ..... 61.5% (35.2% acid equivalent)	Transvaal
Verton 2D[d]	Propylene glycol butyl ether esters of 2,4-D ..... 39.6% (24.5% acid equivalent) in xylene	Dow

Table 1-2 (Concluded)

Formulation	Active Ingredients	Manufacturer[b]
Visko-Rhap 2,4-D Low Volatile Ester 4L	Isooctyl ester of 2,4-D 67.75%	Rhodia, Chipman Division[c]
Visko-Rhap Low Volatile Ester 2D	2-Ethylhexyl ester of 2,4-D (isooctyl ester) 36.8%, 37.1%	Rhodia, Chipman Division[c]
Visko-Rhap Oil-Soluble Amine A-3D	N,N-Dimethyloleyl-linoleyl amine salt of 2,4-D 63.86%	Rhodia, Chipman Division[c]
Weedar 64	Dimethylamine salt of 2,4-D 49.5%	Amchem
Weedar 64A	Dimethylamine salt of 2,4-D 57.4%	Amchem
Weedar 96	Dimethylamine salt of 2,4-D 69.7%	Amchem
Weed-Rhap LV-6D	2-Ethylhexyl ester of 2,4-D ..... 94.2% (62.5% acid equivalent)	Transvaal

[a] Formulations with herbicide other than 2,4-D are omitted.

[b] Use of trade names and company names is only to provide information and does not imply product endorsement.

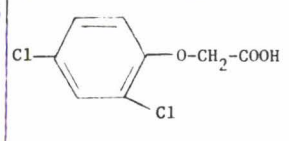
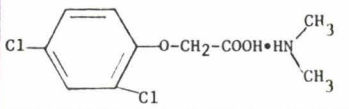
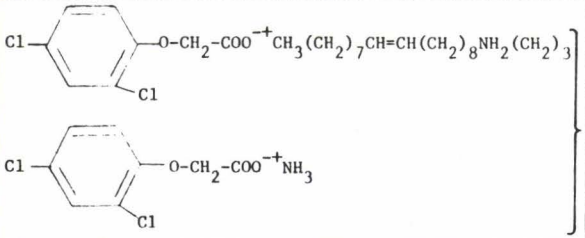
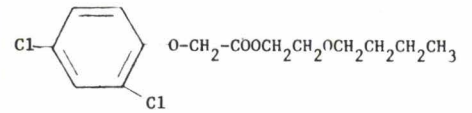
[c] Rhodia no longer produces phenoxy herbicides.

[d] No longer available.

Source: Gosselin et al. 1976.

Table 1-3

Selected Chemical and Physical Properties of 2,4-D and Two of Its Salts and a Low-Volatile Ester

Name	Structural Formula	Molecular Formula (Molecular Weight)	Vapor Pressure	Solubility
(2,4-dichlorophenoxy) acetic acid		$C_8H_6Cl_2O_3$ (221.0)	0.4 mmHg (320°F, 160°C)	0.09% in water (77°F, 25°C) 85.0% in acetone (77°F, 25°C) 0.1 to 0.35% in kerosene and diesel oil (77°F, 25°C) 130% in ethyl alcohol (95%; 77°F, 25°C)
Dimethylamine salt of 2,4-D		$C_{10}H_{13}Cl_2NO_3$ (266.1)	NA[a]	300% in water (68°F, 20°C) Soluble in acetone and in methyl, ethyl and isopropyl alcohols. Insoluble in kerosene and diesel oil.
N-oleyl-1,3-propylenediamine salt of 2,4-D		$C_{37}H_{56}Cl_4N_2O_6$ (766.6)	NA[a]	Insoluble in water, soluble in most organic solvents.
Butoxyethanol ester of 2,4-D		$C_{14}H_{18}Cl_2O_4$ (321.2)	Less than 1.5×10^{-4} mmHg (77°F, 25°C), estimated	Insoluble in water, soluble in most organic solvents.

[a]Not available.

Source: Loos 1975 and Weed Science Society of America 1983.

(25°C). Consequently, formulations derived from such low-molecular-weight alcohols tend to volatilize readily and pose a potential toxic hazard to nearby susceptible nontarget crops. The introduction of esters based on high-molecular-weight alcohols, such as the 2-ethylhexyl, isooctyl, butoxyethyl, propylene glycol butyl ether, and tetrahydrofurfural esters of 2,4-D, with estimated vapor pressures less than 1.5×10^{-4} mm Hg at 77°F (25°C), has overcome the problem of volatility hazard to sensitive nontarget crops (Loos 1975).

Various forms of 2,4-D are degraded by both biological and nonbiological mechanisms. Degradation of 2,4-D is reviewed extensively in Loos (1975) as well as in Ghassemi et al. (1981), Norris (1981), Lommen (1980), Mullison (1981), and Weed Science Society of America (1983). In soil, 2,4-D is subject to loss by volatilization as well as by chemical and biological degradation. Volatilization rates depend to a large extent on the specific 2,4-D formulations. Acid, inorganic salt, and amine formulations are generally less volatile than ester formulations, which in turn range from low to high volatility (Weed Science Society of America 1983). Soil microorganisms readily metabolize 2,4-D, with a higher rate of 2,4-D degradation occurring under conditions of warmth and moisture that also promote growth of microorganism populations (Weed Science Society of America 1983). In animals, 2,4-D esters are readily hydrolyzed to the free acid form which is in turn eliminated, primarily in the urine. In plants, 2,4-D may be metabolized, resulting in degradation of the side chain, hydroxylation of the ring structure (addition of OH^- to the ring structure of 2,4-D), conjugation of 2,4-D with plant constituents such as glucose or aspartic acid, formation of various metabolites, or, in some cases, ring cleavage or side-chain lengthening (Loos 1975). Photodecomposition is a minor factor in the loss of 2,4-D from soil (Weed Science Society of America 1983).

2.0 Toxicity of 2,4-D

The toxicological properties of 2,4-D, summarized in Table 2-1, indicate that most formulations of 2,4-D are mildly toxic to mammals and birds. Most ester formulations are toxic to highly toxic to aquatic invertebrates and to fish, while salt and acid formulations are generally only slightly toxic to these organisms. Some formulations, such as salts, esters, and concentrated solutions, are eye irritants that can cause injury. Some formulations may cause irritation to the skin (Mullison 1981 and Weed Science Society of America 1983). Inhalation toxicity is minimal. There are no conclusive data demonstrating the carcinogenicity or teratogenicity of 2,4-D. However, several studies have indicated embryotoxic and fetotoxic effects of some formulations of 2,4-D.

Due to the large body of literature on 2,4-D toxicity generated since its introduction as a herbicide in the mid-1940's, the following discussions of 2,4-D toxicity in animal species are based primarily on studies that have been conducted within the past 20 years. Various reviews on the toxicity of 2,4-D that incorporate earlier studies include Mullison (1981), Pimentel (1971), Minnesota Department of Health (1978), Lommen (1980), Ghassemi et al. (1981), and the International Agency for Research on Cancer (1977).

2.1 Invertebrates and Microorganisms

Toxicity of 2,4-D (technical acid) and many of its formulations have been determined for a wide variety of invertebrate species and microorganisms from terrestrial, marine, and freshwater ecosystems. Table 2-2 is a comparison of the ranges of observed toxic effects for 2,4-D (technical, acid, or formulation unspecified), for salts of 2,4-D, and for esters of 2,4-D in insects, crustaceans, other invertebrates, and microorganisms. In general, for aquatic organisms, lethal effects (LC_{50}'s) at concentrations below 1 ppm are considered indicative of highly toxic substances, effects at concentrations of 1 to 10 ppm are considered indicative of toxic substances, and effects at concentrations above 10 ppm are considered indicative of slightly toxic compounds (Clarke et al. 1970). The toxicity data presented in Appendix A and compared in Table 2-2 indicate a wide range of toxicities, from highly toxic to slightly toxic, for 2,4-D, its salts, and its esters in most invertebrate and microorganism groups. This variation is a function of real differences in chemical toxicities of the 2,4-D formulations to different species as well as numerous other factors. These include experimental and environmental variability; water temperatures and chemistry; test organism nutritional status, sex, and life stage; photoperiod; season; and flow dynamics of test apparatus. In considering these data, it should also be noted that, for the most

Table 2-1

Summary of Toxicological Properties of 2,4-D

1. Acute Oral Toxicity

Organism	Formulation	Adverse Effects
Mouse	2,4-D (acid)	LD_{50} = 368 to 375 mg/kg
Rat		LD_{50} = 375 to 666 mg/kg
Guinea pig		LD_{50} < 320 to 1,000 mg/kg
Rabbit		LD_{50} = 800 mg/kg
Dog		LD_{50} = 100 mg/kg
Chick		LD_{50} = 541 mg/kg
Mule deer		LD_{50} = 400 to 800 mg/kg
Mouse	2,4-D (butyl ester)	LD_{50} = 380 to 731 mg/kg
Rat		LD_{50} = 620 to 1,500 mg/kg
Guinea pig		LD_{50} = 848 mg/kg
Rabbit		LD_{50} = 424 mg/kg
Chick		LD_{50} = 2,000 mg/kg
Cattle		LD_{50} <100 mg/kg
Cats		LD_{50} = 820 mg/kg
Mouse	2,4-D (isopropyl	LD_{50} = 541 mg/kg
Rat	ester)	LD_{50} = 700 mg/kg
Guinea pig		LD_{50} = 550 mg/kg
Chicks		LD_{50} = 1,420 mg/kg
Chicken	2,4-D (amines)	LD_{50} = 380 to 765 mg/kg
Sheep		Adverse effects at doses 250 mg/kg
Cattle		Adverse effects at doses 50 mg/kg
Pig		Adverse effects at doses 50 mg/kg
Mouse	2,4-D (sodium salt)	LD_{50} = 360 to 375 mg/kg
Rat		LD_{50} = 666 to 2,000 mg/kg
Guinea pig		LD_{50} = 551 to 1,000 mg/kg
Rabbit		LD_{50} = 800 mg/kg
Chicken		LD_{50} = 655 mg/kg
Monkey	2,4-D (unspecified)	Adverse effects at doses 428 mg/kg

Table 2-1 (Continued)

2. Acute Dermal Toxicity

Organism	Formulation	Adverse Effects
Rat	2,4-D (unspecified)	LD_{50} = 1,500 mg/kg
Rabbit		LD_{50} = 1,400 mg/kg
Human		Skin irritant
Rabbit	2,4-D (amine and esters)	Subepithelial fibrosis and mononuclear infiltration.

3. Eye Irritation

Organism	Formulation	Adverse Effects
(unspecified species)	2,4-D DMA®4, Formula 40®, Esteron 99®, concentrated solution, or wetted material	Eye irritation involving conjunctival membranes and cornea.

4. Inhalation Toxicity

Organism	Formulation	Adverse Effects
Human	2,4-D (unspecified)	Airborne TLV = 10 mg/m^3; TLV short-term exposure limit = 20 mg/m^3. Can cause coughing, burning, dizziness, and muscle incoordination.
Guinea pig	2,4-D (sodium salt)	No adverse systemic effects.

5. Subchronic Oral Toxicity

Organism	Formulation	Length of Treatment	Dose Tested	Adverse Effects
Mouse	2,4-D (sodium salt)	3 to 12 weeks	93 mg/kg daily in diet	No adverse effects.
Rat	2,4-D (unspecified)	1 month	1,000 ppm in diet	No adverse effects.
Rat	2,4-D (unspecified)	1 month	0 to 300 mg/kg by intubation	Gastrointestinal irritation and depressed growth rate.

Table 2-1 (Continued)

5. Subchronic Oral Toxicity (Concluded)

Organism	Formulation	Length of Treatment	Dose Tested	Adverse Effects
Rat	2,4-D (sodium salt)	1 month	0 to 500 mg/kg in diet	Death at 500 mg/kg.
Rat	2,4-D (amine)	113 days	0 to 10,000 ppm in diet	Weight loss, liver and kidney damage, death.
Rat	2,4-D (amine)	10 months	1,000 ppm in diet	No adverse effects.
Guinea pig	2,4-D (unspecified)	12 days	10 doses of 100 mg/kg	No adverse effects.
Dog	2,4-D (unspecified)	90 days	Fed 0 to 20 mg/kg/day for 5 days/week by capsule	Difficulty swallowing, bleeding gums, stiff legs, liver and kidney damage, and death.
Chicken	2,4-D (unspecified)	7 days	0 to 3,000 ppm in diet	Retarded growth rate.
Chicken	2,4-D (amine)	10 days	100 mg/kg/day orally	No adverse effects.
Chicken	2,4-D (amine)	24 days	300 mg/kg/day orally	Renal and visceral gout, enlarged kidney, and death.
Chicken	2,4-D (unspecified)	1 day old to maturity	1,000 ppm in drinking water	Reduced egg production, increased weight, and epithelial hyperplasia of kidney.
Cattle	2,4-D (amine and ester)	112 days	50 mg/kg/day orally	No adverse effects.
Sheep	2,4-D (amine and ester)	481 days	100 mg/kg/day orally	Weight loss.
Pig	2,4-D (amine and ester)	103 days	50 to 300 mg/kg/day	Anorexia, retarded growth, locomotion disturbances, decreased hemoglobin, hematocrit, and albumin.
Pig	2,4-D (amine and	12 months	500 ppm in diet	

Table 2-1 (Continued)

6. Abdominal Toxicity/Subchronic Intravenous

Organism	Formulation	Length of Treatment	Dose Tested	Adverse Effects
Dog	2,4-D (sodium salt)	32 to 37 days	1.17, 2.6 and 3.2 mg/kg/day	No adverse effects.
Dog	2,4-D (unspecified)	6 days	25 mg/kg/day	Liver damage.
Human	2,4-D (unspecified)	32 days	16.3 gm total dose	Stupor, incoordination, weak reflexes, and urinary incontinence.

7. Subchronic Dermal Toxicity

Organism	Formulation	Length of Treatment	Dose Tested	Adverse Effects
Rabbit	2,4-D (amines and esters)	up to 3 weeks	up to 3,980 mg/kg	Local skin inflammation; LD_{50} = 3,980 mg/kg

8. Chronic Toxicity

Organism	Formulation	Length of Treatment	Dose Tested	Adverse Effects
Mouse	2,4-D (acid and esters)	Single subcutaneous injection and observed 78 weeks	Varied with formulation from 21.5 to 215 mg/kg.	Reduced survival; reticulum cell sarcomas.
Rat	2,4-D (acid)	2 years	1,000 ppm in diet	No adverse effects.
Chicken	2,4-D (amine)	18 months	500 ppm in diet	Renal gout, death, muscle degeneration, stomach ulcer, anemia, and lymphocytic peribronchitis
Dog	2,4-D (96.6% pure)	2 years	0 to 500 ppm in diet	Death

Table 2-1 (Continued)

9. Teratology, Reproduction and Fertility

Organism	Formulation	Dose and Length of Treatment	Adverse Effects
Mouse	2,4-D (acid and esters)	Treated orally or subcutaneously on days 6-14 of pregnancy. Doses unspecified.	Increased incidence of fetal abnormalities.
Rat	2,4-D (unspecified)	Doses up to 188 mg/kg during pregnancy. Route unspecified.	Not teratogenic. Decreased body weight and size of fetuses. Abdominal bleeding. Increased intrauterine mortalities.
Rat, Sprague-Dawley	2,4-D	Fed 1,000 ppm in drinking water during pregnancy, an additional 10 months to mother, and an additional 2 years to offspring.	Reduced food consumption, water consumption, and growth rate in offspring maintained on treatment. No adverse clinical signs; no malformations; normal pregnancy, birth, and litter sizes.
Rat, Wistar	2,4-D (acid, amine and esters)	Single oral daily doses of 0 to 150 mg/kg on days 6-15 of pregnancy. Sacrificed on day 22.	Embryotoxic, fetopathic, and potentially teratogenic. Decreased litter size, fetal weight, and newborn survival. Increased skeletal abnormalities (e.g. wavy ribs, and extra ribs, delayed ossification).
Rat, Sprague Drawley	2,4-D (acid and ester)	Intubation of doses up to 142 mg/kg/day on days 6-15 of pregnancy.	Embryotoxic and fetotoxic but not teratogenic. Esters decreased litter size and newborn survival rate. Observed edema, delayed ossification, wavy ribs, and lumbar ribs.
Hamster Syrian-Golden	2,4-D (acid and esters)	Intubation of 20 to 100 mg/kg/day on days 6-10 of pregnancy. Sacrificed on day 14.	Not teratogenic. Decreased fetal viability.
Sheep	2,4-D (unspecified)	Intubation of 40 mg/kg daily for 30, 60, and 90 days following breeding.	No birth defects and no clinical or histopathological signs of toxicity.

Table 2-1 (Continued)

9. Teratology, Reproduction and Fertility (Concluded)

Organism	Formulation	Dose and Length of Treatment	Adverse Effects
Sow	2,4-D (unspecified)	Fed 500 ppm in diet during pregnancy. Piglet fed 500 ppm in diet for 8 months.	Ten of 15 piglets died. Surviving piglets had depressed growth, anemia, and degenerative changes in liver and kidney.

10. Carcinogenicity

Organism	Formulation	Dose and Length of Treatment	Adverse Effects
Mouse	2,4-D (amine and esters)	Dermal application of 2 drops of 10% solution or intubation of doses up to 100 mg/kg followed by diets up to 149 ppm for 18 months.	Not carcinogenic. Not tumorigenic.
Rat	2,4-D (amine)	Lifetime feeding of doses up to 0.1 x LD_{50}	Not carcinogenic.
Dog	2,4-D (acid)	0 to 500 ppm in diet for 2 years	Not carcinogenic; one death.

11. Mutagenicity

Organism	Adverse Effects
Salmonella typhimurium (2,4-D)	Nonmutagenic when tested at concentrations up to 5,000 μg/plate in the absence or presence of metabolic activation or in host-mediated assays.
Bacillus subtilis (2,4-D)	Nonmutagenic in the Rec assay or in differential toxicity assays employing DNA-proficient or DNA-deficient strains.
Escherichia coli (2,4-D)	Nonmutagenic when tested in forward mutation, reverse mutation, or differential toxicity assays.
Saccharomyces cerevisiae	Mutagenic. Reverse mutation to (2,4-D) histidine independence or mitotic gene conversion and mitotic recombination observed only at low pH (4.5 and below).
Serratia macescens (2,4-D)	Nonmutagenic.
Bacteriophage (E. coli T_4) (2,4-D)	Nonmutagenic.

Table 2-1 (Concluded)

11. Mutagenicity (Concluded)

Organism	Adverse Effects
Blue-green algae (2,4-D)	Nonmutagenic.
Drosophila melanogaster (2,4-D)	Weakly mutagenic. Increased numbers of recessive lethals at very high doses (1,000 ppm). Increased frequency of somatic mutations in unstable but not in stable stream of fruit flies.
Rat hepatocytes (2,4-D)	No induction of UDS.
Human lymphocytes (2,4-D)	Decreased chromosome aberrations and ester chromatid exchanges observed generally at doses of 50 to 60 ppm or above.
Mouse bone marrow cells (2,4-D)	Noncytogenetic to bone marrow erythrocytes but increased chromosome aberrations observed in bone marrow cells of mice treated with 100 to 300 mg/kg.
Rat bone marrow cells (2,4-D)	No chromosomal aberrations.
Mouse spleen cells (2,4-D)	Inhibition of Gamma and UV-induced DNA repair synthesis.
Embryonic bovine kidney (EBK) cells and bovine peripheral blood (BPB) cells (2,4-D)	No chromosomal damage. In EBK cells 2,4-D may have affected spindle protein and cell division.
Mouse (2,4-D)	Nonmutagenic. No increase in dominant lethal mutations.
Fava beans, Vicia faba (2,4-D)	Chromosome aberrations observed in pollen mother cells following spraying of plants or soaking of seeds in 2,4-D.
Barley seeds (2,4-D)	Increased chromosomal aberrations and chlorophyl-deficient mutants.
Tobacco plant cells in culture (2,4-D)	Increased chromosome aberrations.
Various cultivated planes and weeds (2,4-D)	Increased chromosome aberrations.

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

Table 2-2

Comparison of Toxic Effects of 2,4-D Formulations For Selected Invertebrate and Microorganism Taxa

Taxonomic Group	Type of Formulation	Range of Effects[a]
Insects	2,4-D (technical, acid, or no formulation specified)	LD_{50} = 11.5 μg/honeybee. No significant effects on reproduction through three generations in cockroaches fed 1,000 ppm 2,4-D in diet.
	Salts	Seventy percent mortality of mayfly nymphs after 1 hour in 100 ppm dimethylamine. Twenty-four hour IC_{50} = 1,490 ppm for planktonic dipteran larvae at 59°F in 2,4-D amine.
	Esters	Larval midges exposed to 1 ppm butoxyethanol ester had greater mortality than controls. Honeybees fed up to 1,000 ppm butoxyethanol ester or isooctyl ester in diet had same longevity as controls.
Crustaceans	2,4-D (technical, acid, or no formulation specified)	Forty-eight hour TL_{50} = 3.2 ppm for scuds (Gammarus sp.). Ninety-six hour LC_{50} = 417.8 ppm for Daphnia.
	Salts	Forty-eight hour EC_{50} = 0.15 ppm dimethylamine salt for mature glass shrimp. Ninety-six hour LC_{50} = 1,389 ppm alkanolamine salt for immature crayfish.
	Esters	Forty-eight hour TL_{50} = 0.1 ppm propylene glycol butyl ether ester for Daphnia. Forty-eight hour LC_{50} = 100 ppm propylene glycol butyl ether ester or butoxy ethanol ester for crayfish.

Table 2-2 (Concluded)

Taxonomic Group	Type of Formulation	Range of Effects
Invertebrates other than Insects or Crustacea	2,4-D (technical, acid, or no formulation specified)	No effect on shell growth in oysters after 96 hours in 2 ppm 2,4-D. Ninety-six hour LC_{50} = 122.2 ppm for freshwater oligochaete worm with no toxic effects at 86.7 ppm.
	Salts	No effect on shell growth in oysters after 96-hour in 2 ppm dimethylamine salt.
	Esters	Reproductive impairment in freshwater ectoprocts in 0.01 ppm butyl ester after 84 hours. Thirty-eight percent reduction in shell growth in oysters after 96 hours in 5.0 ppm 2-ethylhexyl ester.
Microorganisms	2,4-D (technical, acid, or no formulation specified)	Euglena cultures were not adversely affected after 3 days in 220 ppm 2,4-D.
	Salts	No decrease in carbon fixation was observed in phytoplankton after 4 hours in 1.0 ppm dimethylamine salt.
	Esters	Forty-nine percent decrease in carbon fixation was observed in phytoplankton after 4 hours in 1.0 ppm 2-ethyl hexyl ester. Ten percent of planktonic algal cultures showed inhibited growth response after 2 weeks in 4.0 ppm butoxy ethanol ester.

[a]Data abstracted from Appendix A. See Appendix A for references.

part, the studies on toxicity of 2,4-D and its formulations to invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of 2,4-D or its formulations in the natural environment. For example, in the laboratory, aquatic organisms were kept for as long as 69 days with continuous exposure to 2,4-D. Microorganisms were grown in culture medium containing 2,4-D, often at concentrations several orders of magnitude greater than those normally found in the environment following application. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to 2,4-D or other man-made chemicals is usually a transient phenomenon whose duration depends on the rate of streamflow and on the rate of chemical decomposition of the substance. Additionally, in natural systems, interaction with other man-made and naturally occurring chemicals and changes in environmental conditions may affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions, in turn, potentially alter the tolerance levels of organisms to the herbicides.

The following subsections review the data on comparative toxicity of 2,4-D and its formulations to insects, crustaceans, other invertebrates, and microorganisms.

2.1.1 Insects

Insects appear to be relatively tolerant to high levels of 2,4-D. The few studies in which median lethal concentrations on the order of 1 ppm were observed were with 2,4-D ester formulations. Honeybees, representing insects of commercial importance, show a tolerance to 2,4-D, which is considered a stomach and contact poison with low toxicity to honeybees (Glynne Jones and Connell 1954 in Way 1969). Bees directly dosed with 2,4-D varied in response from an LD_{50} of 11.525 μg/bee for an unspecified route of exposure (Beran and Neururer 1955 in Pimental 1971) to an oral LD_{50} of 105 μg/bee (Glynne Jones and Connell 1954 in Way 1969). Bees fed up to 1,000 ppm of the butoxyethanol ester, isooctyl ester, or the dimethylamine salt of 2,4-D had half-lives (length of time for half of the bees in the colony to die) as long or longer than control bees (Morton et al. 1972). On the other hand, bees fed purified 2,4-D had a shorter life span when fed 1,000 ppm than controls, but not when fed 100 ppm (Morton et al. 1972). Bees fed 100 ppm or more of an unspecified 2,4-D formulation (presumed here to be an acid) had a temporary (reversible) decrease in reproductive rate (brood production) that did not appear when bees were fed 10 ppm 2,4-D (Morton and Moffett 1972).

Lichtenstein et al. (1973) found application of 10 to 40 μg 2,4-D (analytical grade) per bioassay jar to potentiate the toxic effects of insecticides in some insects. They observed a signficant increase in the toxicity of DDT to fruit flies exposed to 2,4-D and in the toxicity of parathion to mosquito larvae exposed to 2,4-D.

2.1.2 Crustaceans

Studies with crustacea indicate that 2,4-D technical and acid formulations are toxic or slightly toxic to most species, that salt formulations are slightly toxic to most species, and that ester formulations are toxic to highly toxic to most species (see Table 2-2 and Appendix A for specific results).

Several studies indicated sublethal effects of 2,4-D and formulations to crustacea. For instance, Caldwell (1977) and Caldwell et al. (1979) found chronic exposure of larval dungeness crabs to concentrations of 2,4-D as low as 1 ppm to result in delayed molting, while juvenile molting was advanced in crabs exposed to 100 ppm. Sigmon (1979a) found a higher rate of respiration in adult Daphnia exposed to 3 ppm of the butoxyethanol ester of 2,4-D than controls, although this difference was not statistically significant.

2.1.3 Invertebrates Other than Insects or Crustaceans

In addition to insects and crustaceans, toxic effects of 2,4-D have been reported for invertebrates such as molluscs (oysters), annelids (freshwater oligochaete worms), rotifers, and several species of freshwater ectoprocts. For the Eastern oyster, Butler (1965) reported 39 percent reduction in shell growth (compared to controls) at 1.0 ppm propylene glycol butyl ether ester of 2,4-D; no effect at 2.0 ppm 2,4-D acid or dimethylamine salt of 2,4-D; 38 percent reduction in shell growth at 5.0 ppm 2-ethyl hexyl ester of 2,4-D; and an EC_{50} of 375 ppm for butoxy ethanol ester of 2,4-D during 96-hour bioassays. A freshwater oligochaete worm (Lumbriculus sp.) had a 96-hour LC_{50} of 122.5 ppm 2,4-D acid with no toxic effects observed at a concentration of 86.7 ppm (Bailey and Liu 1980). Rotifers (Brachionus sp.) tested in 5 ppm 2,4-D were all dead within 31 hours and had 50 percent mortality within 24 hours (George et al. 1982). Rao and Dad (1979) tested three genera of freshwater ectoprocts in the butyl ester of 2,4-D and reported 100 percent mortality in all three after 48 hours at 4 ppm 2,4-D.

2.1.4 Microorganisms

Microorganisms, including soil fungi and amoeba, blue-green algae, and planktonic algae have been grown in cultures of 2,4-D or its salts and esters. The growth of soil fungi in laboratory

cultures of 2,4-D (specific formulations not reported in some studies, reported as acid in others) was in enhanced at levels as high as 15 ppm (Moubasher et al. 1981). Fungal growth was stimulated in agar at 6.3 ppm, for some species, and toxic to all species at 25.2 ppm (Moubasher et al. 1981). The population growth or metabolism of bacteria and fungi was unaffected at 10 ppm (Tu 1979), but the growth and reproduction of a soil amoeba were stimulated at concentrations as high as 100 ppm (Prescott and Olson 1972). Varying effects, ranging from inhibition to enhancement of growth, were observed in soil microorganisms grown in soil cultures with 2,4-D incorporated at a rate equivalent to 10,000 lbs/acre (Stojanovic et al. 1972). All of 23 strains of free-living amoeba were unaffected when grown in cultures with up to 84 ppm of an unspecified formulation of 2,4-D (Pons and Pussard 1980). Concentrations up to 100 ppm 2,4-D (formulation not specified) incorporated into culture medium did not affect metabolism as measured by oxygen consumption of sewage sludge microorganisms in a culture derived from sewage organic matter (Lieberman and Alexander 1981 and Rosenberg et al. 1979).

Algae have been grown in cultures containing unspecified formulations of 2,4-D as acid, salts, or esters. Butler (1965) reported no decrease in carbon fixation for phytoplankton cultures following 4-hour exposure to 1.0 ppm 2,4-D acid or to 1.0 ppm of the dimethylamine salt of 2,4-D. Exposure of phytoplankton for 4 hours to 1.0 ppm of the butoxyethanol ester of 2,4-D resulted in a 16 percent reduction in carbon fixation. Exposure to the 2-ethyl hexyl ester of 2,4-D resulted in a 49 percent reduction in carbon fixation, and exposure to the propylene glycol butyl ether ester produced a 44 percent reduction in carbon fixation compared to controls (Butler 1975). Euglena, cultured for 3 days in 2,4-D (99 percent) at a concentration of 220 ppm, were unaffected in terms of motility or population growth (Morre 1974 and Elder et al. 1970). However, the growth rate of cyanobacteria (blue-green algae) cultured in 2,4-D (unspecified formulation) was reduced at concentrations as low as 50 ppm, and population growth was completely inhibited at 100 ppm for some cultures (Hutber et al. 1979). For planktonic algae cultured 2 weeks in media with the butoxyethanol ester of 2,4-D, concentrations of 1 ppm or less did not change algal population growth patterns, while 10 percent of the cultures had inhibited growth responses when cultured in 4.0 ppm of the ester formulation (Butler et al. 1975b).

2.2 Fish and Amphibians

Recent studies (primarily reported within the past 20 years) on the toxicity of 2,4-D and its formulations to fish and amphibians are summarized in Appendix B. In most cases, fish LC_{50}'s were reported to be greater than 1.0 ppm and, in many instances, greater than 10 ppm. LC_{50}'s less than 1.0 ppm were reported in various fish species

tested in the n-oleyl-1,3-propylenediamine salt of 2,4-D (Johnson and Finley 1980), some species tested in the butyl ester of 2,4-D (Johnson and Finley 1980; Meehan et al. 1974; and Woodward 1982), and some species tested in the propylene glycol butyl ether ester of 2,4-D (Johnson and Finley 1980).

Clarke et al. (1970) suggest that pesticides with median lethal concentrations of 10 ppm or more for fish be considered "slightly toxic," between 1 and 10 ppm be considered "toxic," and less than 1 ppm be considered "highly toxic." Thus, 2,4-D technical or acid formulations should be considered toxic to slightly toxic to fish. With the exception of n-oleyl-1,3-propylenediamine, which should be considered highly toxic, organic salts of 2,4-D should be considered toxic to slightly toxic to fish. Inorganic salts of 2,4-D such as potassium, sodium, and lithium salts tend to be only slightly toxic to fish, with LC_{50}'s generally greater than 10 ppm. Esters of 2,4-D generally have LC_{50}'s of 1 ppm or less and should be considered toxic to fish. Table 2-3 provides a comparison of median lethal concentrations of various formulations of 2,4-D to bluegill sunfish and indicates not only the variability in lethal effects of different formulations of 2,4-D, but also the variability in different batches of the same formulation. Table 2-4 summarizes the extreme ranges of lethal effects of selected 2,4-D formulations to fish derived from the data in Appendix B.

In addition to lethal effects of 2,4-D and its formulations, various studies with fish have noted varied sublethal effects, often manifested at concentration levels well below those that would produce death. The addition of 1 ppm of the insecticide carbaryl (a nonlethal concentration) to the butyl ester of 2,4-D potentiated toxic responses of the 2,4-D formulation in rainbow trout (Statham and Lech 1975). Addition of carbaryl resulted in mortality at concentrations of 2,4-D which, by itself did not result in death. The addition of carbaryl also increased the rate of fish mortality when added to lethal concentrations of 2,4-D. Impaired swimming performance was observed in green sunfish within 1 hour after exposure to 110 ppm (a.e.) of the butoxyethanol ester of 2,4-D (Morre 1974, Sergeant et al. 1971 and Sargent et al. 1970 [the latter two references are to the same material and authors, but different spellings of the name of the first author]). Sockeye salmon juveniles showed stress responses in the form of elevated blood plasma cortisol after 1-hour exposure to 5 ppm of the butoxyethanol ester of 2,4-D in August and September, but not in June. The minimal concentration required to induce a stress response was reported to be only marginally below lethal levels of 2,4-D (McBride et al. 1981). Rainbow trout exposed to low concentrations of the dimethylamine salt of 2,4-D in a Y-maze displayed avoidance reactions to concentrations of 2,4-D as low as 1 ppm (Folmar 1976, 1878).

Table 2-3

Comparison of LC_{50}'s for Bluegill Exposed to Different Formulations of 2,4-D

Formulation of 2,4-D[a]	LC_{50} (Acid Equivalent in PPM)	
	24-Hour	48-Hour
Alkanolamine, ethanol and isopropanol series	900	840
Alkanolamine, ethanol and isopropanol series	588	530
Alkanolamine, ethanol and isopropanol series	450	435
Dimethylamine	542	458
Dimethylamine	500	416
Dimethylamine	390	353
Dimethylamine	273	273
Dimethylamine	220	220
Dimethylamine	166	166
Di-n,-v-dimethylcocoamine	1.5	1.5
2,4-D acid, with emulsifiers	8.0	8.0
Isooctyl ester	66.3	59.7
Isooctyl ester	36.0	36.0
Isooctyl ester	8.8	8.8
Propylene glycol butyl ether ester	2.1	2.1
Butoxyethanol ester	2.1	2.1
Butyl ester	1.3	1.3
Mixed butyl and isopropyl esters	1.7	1.7
Mixed butyl and isopropyl esters	1.6	1.5
Isopropyl ester	0.9	0.8
Ethyl ester	1.4	1.4

[a]Includes different batches of the same formulation.

Source: Hughes and Davis 1963 in Pimentel 1971.

Table 2-4

Extreme Median Lethal Concentrations of Selected 2,4-D Formulations in Fish

Formulation	Observed Median Lethal Concentrations (LC_{50})		References[a]
	Lowest	Highest	
2,4-D (acid)	5.1 ppm (96-hour) Common carp (May)	NOEL at 50 ppm (48-hour) Spot	Vardia and Durve 1981; Butler 1965
Dimethylamine salt	>100 ppm (96 hour) Chinook salmon and Rainbow trout	3,080 ppm (24-hour) 1,313 ppm (96-hour) Grass carp	Johnson and Finley 1980; Tooby et al. 1980
2,4-D (sodium salt)	32.0 ppm (96-hour) Mullet (30-day fry)	NOEL at 100 ppm (8-day) Bluegill (young fish)	Tag El-Din et al. 1981; Hiltibran 1967
Butoxyethanol ester	1.2 ppm (96-hour) Bluegill (1.4 gram)	10.0 ppm (24-hour) Rainbow trout (yearlings)	Johnson and Finley 1980; Dodson and Mayfield 1979
Propylene glycol butyl ether ester	0.6 ppm (96-hour) Bluegill (1.0 gram)	4.5 ppm (48-hour) Long-nosed killifish	Johnson and Finley 1980; Butler 1965
Butyl ester	0.78 ppm (96-hour) Cutthroat trout	$1.0 < LC_{50} < 5.0$ (96-hour) Alaska coho salmon, Dolly Varden char, and Oregon coho salmon (all fingerlings)	Woodward 1982; Meehan et al. 1974

[a]First reference is the source for the lowest LC_{50}, second reference is the source for the highest LC_{50}.

In a summary of toxicity tests conducted at the Columbia, Missouri National Fisheries Research Laboratory, Johnson and Finley (1980) reported on the effects of environmental factors affecting the toxicity of 2,4-D and its formulations to fish. Differences in test-water hardness from 44 to 300 ppm did not have any effect on toxicity of 2,4-D or its formulations. With some formulations, toxicity was affected by pH variations in the range of 6.5 to 8.5. Within this range, toxic effects of 2,4-D acid, butyl ester, and dimethylamine salt were half as toxic at pH 8.5 than at pH 6.5. For the dodecyl/tetradodcyl amine of 2,4-D, toxicity to fathead minnows was 4 times as great at pH 8.5 than at 6.5 (96-hour LC_{50} = 2.4 ppm at pH 8.5; 8.4 ppm at pH 6.5). Several formulations, such as the butyl ester and the propylene glycol butyl ether ester, had reduced fish toxicities when the test solutions were aged prior to testing. Finally, for several 2,4-D formulations, the actual age of the fish at testing affected toxicity levels for the formulation. In general, fry and fingerlings were more sensitive than eggs for rainbow trout tested with dodecyl/tetradodecyl amine salts and for fathead minnows tested with the dimethylamine.

For amphibians, studies have been conducted on the toxic effects of 2,4-D amine on frog and toad tadpoles indicating only slight toxicity, with 96-hour TL_{50}'s of 200 ppm or greater for three species of 1 to 2-week old tadpoles (Johnson 1976). Older tadpoles (4-weeks) of the one species tested at that age (Adelotus brevis) were more resistant than younger individuals, indicating an increased resistance to 2,4-D amine with age.

2.3 Birds

The toxicity of various formulations of 2,4-D to birds has been studied by dietary and oral studies, egg injection bioassays, and by means of solutions sprayed onto eggs. These studies, summarized in Appendix C, indicate that 2,4-D and it formulations have low toxicity to birds.

In general, dietary studies indicated a low toxicity to birds for formulations such as 2,4-D technical, acid, and amine as reported in the literature. Reported acute oral LD_{50}'s range from 472 mg/kg body weight for 2,4-D (technical) administered by capsule to 3 to 4 month old male pheasants, to >>2,025 mg/kg body weight, for 2,4-D sodium salt administered by capsule to 3 to 5 month old male and female mallards (Tucker and Crabtree 1975). Subchronic oral toxicities range from reduced weight gains in chickens fed 10 daily doses of 100 mg propylene glycol butyl ether ester (Palmer and Radeleff 1969) to LC_{50}'s >5,000 ppm for the butoxyethanol ester or the dimethylamine salt of 2,4-D fed to bobwhites, Japanese quail, ring-necked pheasants, or mallards, or for 2,4-D acetamine fed to Japanese quail (Hill et al. 1975).

Injection of 2,4-D (specific formulation not given) in an acetone carrier into fertile chicken eggs to give concentrations of 10 to 300 ppm resulted in reduced hatch (71 percent of controls) at a concentration of 100 ppm 2,4-D (Dunachie and Fletcher 1970). Aqueous solutions of 2,4-D amine, the propylene glycol butyl ether ester of 2,4-D, or the isooctyl ether ester of 2,4-D, sprayed at various concentrations on eggs of chickens and pheasants, caused no adverse effects. The endpoints examined included deaths during early or late incubation, failed pipping of shell, failure of hatching, and abnormal weight gain or mortality rate of successfully hatched offspring (Somers et al. 1973, 1978a,b; Kopischke 1972).

2.4 Mammals

2,4-D is a widely used herbicide that has been intensively studied. The potential health hazards from acute and chronic exposure to 2,4-D have been evaluated in laboratory mammals including rats, mice, rabbits, guinea pigs, dogs, and sheep. Limited data relating effects of human and wild animal exposures are also available. The results of toxicity tests in mammals and the effects of exposure in humans are summarized in Table 2-1 and Appendix D.

Based on acute and chronic toxicity studies, 2,4-D is mildly toxic causing gastrointestinal disturbances, weight loss, muscle weakness, and incoordination. Some formulations cause mild eye, skin, and respiratory irritation. There are no conclusive data demonstrating the carcinogenicity of 2,4-D. There is, however, evidence that 2,4-D is potentially teratogenic. Several studies in laboratory animals also have disclosed embryotoxic and fetotoxic properties of 2,4-D.

The phenoxy herbicides 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) have been combined in some herbicide mixtures. A unique contaminant of 2,4,5-T is TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). The toxic effects of TCDD, namely chloracne and fetal malformations, have attracted considerable public and scientific attention. It should be emphasized that the mammalian toxicology discussed in this report is for 2,4-D and that the 2,4-D formulations do not contain TCDD.

2,4-D has been used for nearly 40 years. A review of early toxicity studies follows. In the first report in 1946 on the effects of 2,4-D in small animals, a single injection of 150 to 250 mg/kg caused muscle spasms in rats, mice, rabbits, and dogs. Repeated injections of 50 to 100 mg/kg for 90 days in mice did not cause muscle spasms. Mice treated with 2,4-D became pregnant and bore normal litters (Bucher 1946). In 1947, Hill and Carlisle published acute oral LD_{50}'s (included in Table 2-1) for various preparations of

2,4-D in small animals. They observed death at highest doses and nausea, vomiting, lethargy, muscle incoordination, paralysis, and stiffness at other doses. All species appeared to respond similarly and the potency between pure and crude preparations, or between sodium and ammonium salts, did not differ significantly (Hill and Carlisle 1947).

Early subchronic toxicity studies using the intravenous, inhalation, and dietary routes of 2,4-D exposure in small animals revealed little evidence of adverse toxic effects (Hill and Carlisle 1947 in Rowe and Hymas 1954). In contrast, early chronic toxicity studies with 2,4-D in rats produced visceral congestion, kidney edema, and kidney tubule degenerative changes. Chronic exposure in dogs resulted in liver damage, degeneration, and congestion (Hill and Carlisle 1947 in Rowe and Hymas 1954). Details of other mammalian toxicity studies spanning nearly four decades are reviewed in Section 2.4.1 through 2.4.6 below.

2.4.1 Acute Toxicity

Acute toxicity data for oral, skin, eye and inhalation exposure are summarized in Table 2-1.

2.4.1.1 Oral. 2,4-D is moderately toxic. The acute oral LD_{50}'s for mice, rats, guinea pigs, rabbits, and mule deer fall in the range of 300 to 1,000 mg/kg. Dogs are more susceptible, with an acute oral LD_{50} of 100 mg/kg. The 2,4-D acid is usually formulated using salts or esters. Dosages in Appendix D are based on the 2,4-D acid content of the formulations. In general, the salts and esters of 2,4-D have approximately the same toxicity as the 2,4-D acid in mammals. Note that the solvent used for oral toxicity testing can greatly influence the toxicity, giving rise in part to the range of LD_{50}'s. For example, the range of LD_{50}'s for the butyl ester of 2,4-D in water is 920 to 1,500 mg/kg and in diesel fuel is 300 to 400 mg/kg (Appendix D). Adverse effects observed in acute toxicity studies include excessive thirst, loss of appetite, loss of weight, depression, roughness of coat, tremors, muscular weakness particularly in posterior quarters, rapid breathing, and salivation (Tucker and Crabtree 1970; Gehring and Betso 1978; and Rowe and Hymas 1954). Post-mortem findings include irritation to the stomach, mild liver and kidney injury, and occasional lung congestion. Muscle spasms and incoordination have been observed following 2,4-D administration under the skin, into the abdominal cavity (e.g. 100 to 250 mg/kg dosage), or intravenously into the blood stream (e.g. 2 mg dosage) (Eyzaguirre et al. 1948 in Mullison 1981).

Approximately 20 cases of 2,4-D toxicity in humans have been reported (U.S. Department of Energy 1983). Toxic signs have resulted from accidental or deliberate ingestion. In an extreme case, an

individual committed suicide by swallowing a 2,4-D formulation. In humans, 2,4-D ingestion or skin exposure can cause irritation to the gastrointestinal tract, chest pain, and muscle twitching (Mullison 1981). An 80 mg/kg dose of the dimethylamine salt of 2,4-D caused congestion in all organs, degenerative nerve cells, and death. In another isolated case reported for humans, accidental swallowing of weed killer containing a dosage of 110 mg/kg of the isooctyl ester of 2,4-D caused muscle twitching and paralysis, although the individual recovered in 24 hours (Mullison 1981). In summary, ingestion of large doses of 2,4-D induces the clinical triad of gastroenteritis, skeletal and cardiac myotonia, and central nervous system depression. The same symptoms are observed in man and experimental animals.

2.4.1.2 Dermal. Administration of various salts and esters of 2,4-D (Table 2-1) result in low acute and subchronic dermal toxicities, with LD_{50}'s from 1,400 to 3,980 mg/kg. Formulations diluted with oil are more likely to cause local inflammatory skin reactions than formulations diluted with water; however the oil itself can be a skin irritant. No adverse systemic effects, such as blood or clinical abnormalities, or adverse neuropathology, are observed following application of 2,4-D formulations to the skin (Mullison 1981). In humans, excessive contact causes skin irritation, tingling of extremities, nausea and vomiting, and muscle aches and loss of function (Mullison 1981 and U.S. Department of Energy 1983).

2.4.1.3 Eye Irritation. The 2,4-D acid as a dry powder is slightly irritating to the conjunctival membranes of the eye. The salt and ester formulations and concentrated solutions of 2,4-D can cause appreciably more eye irritation and potential eye injury (Gehring and Betso 1978 and Rowe 1952 in Mullison 1981). Eye irritation or eye injury are caused by three commonly used 2,4-D formulations available commercially, DMA®4, Formula 40®, and Esteron 99®.

2.4.1.4 Inhalation. 2,4-D is slightly toxic by inhalation exposure. Two Threshold Limit Values (TLV) have been set by the American Conference of Governmental Industrial Hygienists for workers. The airborne TLV is 10 mg/m^3. This is a time weighted average for an 8 hour workday or 40 hour workweek for repeated exposure without adverse effects. The TLV short-term exposure limit is 20 mg/m^3. This is the maximum allowable dose in a 15 minute continuous exposure period. Only four such exposures separated by at least one hour are allowed daily (TLVs 1983). In humans, prolonged breathing of 2,4-D spray causes coughing, burning, dizziness, and temporary loss of muscle coordination (Mullison 1981).

2.4.2 Subchronic Toxicity

Repetitive daily oral dosing of dogs with 20 mg/kg, cattle with 50 mg/kg, and sheep with 100 mg/kg for periods of 10 to 481 days cause no adverse effects. The effects of an amine salt and an ester of 2,4-D were examined in these subchronic studies. Toxic effects observed at higher doses than designated above include loss of appetite, loss of weight, muscular weakness, and gastrointestinal disturbances (Mullison 1981).

Several subchronic toxicity studies have been reported in rats. In rats fed 5 to 500 mg/kg/day for periods up to 113 days, the no observed effect level (NOEL) was 15 mg/kg/day. Depressed growth, excessive mortality, and increased liver weight occurred with 50 mg/kg 2,4-D in the diet. Doses at 150 mg/kg and above were not tolerated. (Rowe and Hymas 1954 in Mullison 1981). In contrast, Hill and Carlisle (1947 in U.S. Department of Energy 1983) reported no adverse effects of doses up to 400 ppm in the diet for 30 days. At 500 ppm, death resulted from feeding the 2,4-D sodium salt. A third rat study reviewed (U.S. Department of Energy 1983) showed no detrimental effects from doses up to 1,000 ppm daily of the 2,4-D amine salt in the diet for 10 months. Subchronic studies are currently in progress to clarify the low but measurable toxicity of 2,4-D formulations.

In humans, ingestion of 8 mg/kg/day for 3 weeks or 21 intravenous (iv) injections of 800 to 960 mg over 32 days caused no adverse effects. Injection of 3,600 mg (equivalent to 51.4 mg/kg/day caused stupor, incoordination, weak reflexes, and urinary incontinence, all of which returned to normal in 24 hours (Mullison 1981).

2.4.3 Chronic Toxicity

Chronic toxicity studies on various 2,4-D formulations are described in chronic toxicity, carcinogenicity and teratogenicity sections of Table 2-1. Both the subcutaneous and oral routes of exposure can cause adverse effects including renal gout, stomach ulcers, anemia, and death. In two specific chronic toxicity studies, rats fed up to 1,250 ppm 2,4-D and dogs fed up to 500 ppm 2,4-D for 2 years were normal clinically and had normal gross and microscopic pathology. In another study, one male dog died at 10 months with no apparent 2,4-D related effects (Hansen et al. 1971 in U.S. Department of Energy 1983).

2.4.4 Carcinogenicity

Based on long-term studies in rats, mice, and dogs with several formulations, 2,4-D is a suspect carcinogen; however, there are no

conclusive data demonstrating the carcinogenicity of 2,4-D (International Agency for Research on Cancer 1977; Mullison 1981; and Minnesota Department of Health, 1978). In one study, rats were fed 0 to 1,250 ppm for 2 years and dogs fed 0 to 500 ppm for 2 years. Only at the highest dose provided male rats was there a significant increase in the total number of animals with malignant tumors. This increase was not significant for any particular tissue. In females, a dose-related increase in both malignant and benign tumors was observed. Because no single dose of 2,4-D in either male or female rats caused a significant increase in a specific tumor type in a specific tissue, no carcinogenicity was originally reported in this rat study. However, a re-evaluation of this study by Dr. Reuber, an independent pathologist, revealed unreported neoplasms. His conclusion was that the tumors were in fact organ specific and that 2,4-D is carcinogenic in rats (Halts 1980 in Lommen 1980). This re-evaluation provides an interpretation of one study that suggests 2,4-D causes cancer in rats; however, this is Dr. Reuber's interpretation and it is not in accord with the conclusions of the original researchers.

In another chronic toxicity study described in Appendix D, mice were given a single subcutaneous injection of 2,4-D and observed until 78 weeks old (International Agency for Research on Cancer 1977). In female mice given 2,4-D isooctyl ester, 5 of 17 developed significantly increased ($p \leq 0.01$) reticulum-cell sarcomas (National Technical Information Service 1968 in Minnesota Department of Health 1978). Dermal application to mice of the amine salt of 2,4-D was not carcinogenic. In other studies in mice, the 2,4-D acid and the isopropyl, n-butyl, and isooctyl esters of 2,4-D were given by stomach tube for 3 weeks and then fed in the diet until terminal sacrifice at 18 months. Both sexes of two mouse strains tested according to this protocal developed no carcinogenic responses to the acid and ester forms of 2,4-D (International Agency for Research on Cancer 1977 and Mullision, 1981). Although no statistically significant increase in tumor incidence of any type in either sex appeared, these studies were declared inadequate. The time span of only 18 months and the group sizes of only 18 mice per group were considered to be an inadequate experimental design in order to allow reliable detection of carcinogenesis even at high doses (Lommen 1980).

The last carcinogenicity study in Appendix D was a 2 year feeding study in dogs. The dogs were fed 0 to 500 ppm 2,4-D acid in their diet and developed no evidence of carcinogenicity. The inability to demonstrate carcinogenicity may have been due to at least two major experimental flaws. Specifically, the experimental duration was too short and there were too few animals to adequately evaluate the carcinogenicity.

In summary, all of the above carcinogenicity data on 2,4-D has been reviewed by the International Agency for Research on Cancer (IARC). The IARC have concluded that the existing data in animals had limitations due either to inadequate reporting or to the small number of animals tested. Although increased tumor incidences were observed in rats treated orally with 2,4-D and in mice treated by subcutaneous injection with the isoctyl ester of 2,4-D, no evaluation of the carcinogenicity of this compound could be made (International Agency for Research on Cancer, 1977).

2.4.5 Reproductive and Teratogenic Effects

Studies on sheep, rats, and hamsters, described in Table 2-1, indicate 2,4-D causes few teratogenic or adverse reproductive effects. Subtle reproductive effects such as decreased birth weight, decreased litter size, or decreased fertility often occur in dosage ranges that cause adult (maternal) toxicity. It is possible that part of the teratogenic potential attributed to 2,4-D may be due to as yet unidentified impurities (Khera and McKinley 1972).

Administration of 40 mg/kg 2,4-D to sheep resulted in no birth defects and no histopathological lesions in lambs. In two Russian studies, no teratogenic effects were induced. However, administration of 188 mg/kg 2,4-D during pregnancy caused decreased body weight, increased intrauterine death rate, enlarged fetal brain cavity, and bleeding in the fetal abdominal cavity (Aleksashina et al. 1973 in Mullison 1981). The study that provides some evidence for the teratogenic potential for the 2,4-D acid, dimethylamine salt, and three ester derivatives was conducted in rats (Khera and McKinley 1972). Doses of 0 to 150 mg/kg of each derivative were administered on days 6 through 15 of pregnancy. The animals were sacrificed on day 22, and the viable fetuses were subsequently examined. The acid and 2,4-D derivatives at high doses (e.g. 100 to 150 mg/kg) caused decreased litter size, decreased fetal weight, and decreased survival of newborne. In addition, all forms of 2,4-D increased the number of skeletal malformations. These included wavy ribs, extra ribs, delayed ossification, and sternum abnormalities. In another study, the 2,4-D acid, dimethylamine salt, and several esters were administered in doses up to 500 mg/kg on days 6 through 15 of pregnancy. The observed effects included fetotoxicity, such as decreased litter size, and decreased newborne survival. At higher doses, an increased number of skeletal anomalies occurred, but 2,4-D and its formulations induced no true teratogenic effect.

One of the early studies to evaluate reproductive effects was done with the 2,4-D amine in pregnant sows (Bjorklund and Ernz 1966). The amine was fed at 500 ppm through pregnancy, then

continued in the food of surviving offspring from 2 months of age. During a protracted parturition, one mummified fetus and 15 piglets ranging from 380 to 1,090 g were born. The piglets were anemic, underdeveloped, apathetic, and would not suck. Ten piglets (2 males and 8 females) died during the first day. Histopathology revealed embryonic hematopoietic foci in the livers. The surviving 5 piglets, placed on 500 ppm diets from 2 months of age until they were 8 months of age, developed retarded growth, locomotor disturbances, fissures and ulcerations of hoofs, depressed hematocrit and hemoglobin values, elevated GOT, and reduced albumin: globulin ratios. Six weeks after parturition, the sow developed hind leg lameness, could not rise, and was killed. Only disc degeneration and abnormalities of the spine were observed. All offspring were sacrificed at 7 to 8 months of age. The organ weights were normal. Multiple disc degenerations were observed. Microscopic pathology revealed kidney tubule dilation, slight degeneration of epithelial cells and parenchyma, and multiple sclerotic foci in spinal cord (in one animal).

Based on the studies in mammals described above, the threshold dose below which no teratogenic response is observed is very high and is expected to be considerably above any potential environmental exposure. Therefore, the levels of 2,4-D used in forestry management are not likely to cause teratogenic responses in humans.

2.4.6 Epidemiology and Human Exposure

Human exposures to 2,4-D do not appear, based on available evidence, to produce carcinogenic or reproductive effects. Epidemiological studies provide the best available sources of information for evaluating health hazards resulting from human exposure to 2,4-D. The effects of isolated exposure to 2,4-D alone are difficult to ascertain because people are exposed to many chemicals over a lifetime and some of these, other than the 2,4-D in question, may be the agent responsible for an adverse effect.

An example of an erroneously interpreted epidemiological study, was conducted in Sweden. A two-fold excess of cancer was reported in Swedish railroad workers exposed to different herbicides compared with the national average (Axelson and Sundell 1970). Evaluation of the data was difficult because of the small number of workers and the concurrent exposure to 2,4-D, amitrole, and 2,4,5-T. The excess cancer may have been due either to exposure to amitrol or to 2,3,7,8-tetrachlorodibenzo-para-dioxin, a known carcinogen and contaminant of 2,4,5-T (Axelson and Sundell 1974 in Lommen 1980 and International Agency for Research on Cancer 1977) rather than to 2,4-D.

In other epidemiological studies, no relationship between 2,4-D exposure and spontaneous abortions (miscarriages) was evident

(Carmelli et al. 1981 in U.S. Department of Energy 1983). These studies were conducted in Washington and Oregon where 2,4-D is extensively used in forests and farmlands.

Several reports of toxic effects experienced by workers in 2,4-D manufacturing plants or in agricultural use (e.g. aerial spraying) are described in Appendix D. The workers were exposed to salts, amines, and esters of 2,4-D for periods from 0.5 to 22 years. Symptoms that developed included weakness, fatigue, headache, dizziness, loss of appetite, nausea, eye irritation, nasopharyngeal irritation, burning sensation of the skin, hypertension, and bradycardia. All symptoms disappeared within days. In one report, long exposure caused liver dysfunction (Bashirov 1969 in Minnesota Department of Health 1978). No adverse clinical effects or chromosomal abnormalities were observed in humans exposed to 2,4-D formulations. The major shortcoming of these human exposure reports is that only cross-sectional data are available. Exposed populations were observed once and occasionally twice. Longitudinal studies, whereby the same exposed individuals are observed at regular intervals over long periods of time, have not been made. Although there is no evidence for 2,4-D carcinogenicity in humans, there are reports that adverse health effects can be produced in humans by acute and chronic exposure to 2,4-D.

2.5 Mutagenicity

The mutagenicity of 2,4-D has been reviewed extensively (Hayes 1982; Mullison 1981; Lommen 1981; Minnesota Department of Health 1978; Seiler 1978; Dost 1978; International Agency for Research on Cancer 1977; and Fahrig 1974). These sources and a number of the original articles which were cited in them were used in this report.

Generally, 2,4-D has been found to be nonmutagenic in most of the microbial systems investigated. For example, 2,4-D was not mutagenic in histidine-dependent *Salmonella typhimurium* mutant strains TA1535, TA1536, TA1537, TA1538, TA98, and TA100, even when some of the tests were performed in the presence of metabolic activation (Anderson et al. 1972; Shirasu 1975; Shirasu et al. 1976; Zetterberg et al. 1977; and Simmon 1979). When tested in a back mutation assay, 2,4-D was not mutagenic to *Serratia marcescens* (Fahrig 1974). Similarly, 2,4-D was not mutagenic to various strains of *Escherichia coli*, either in reverse mutation (Shirasu 1975; Shirasu et al. 1976; and Simmon 1979) or forward mutation assays (Fahrig 1974). However, in a relative toxicity assay with *E. coli*. 2,4-D was more toxic to the DNA repair-deficient strain (p3478) than to the DNA repair-proficient strain (W3110). This occurred only at the highest of the concentrations tested (the range was 0.01 to 5 mg/disc). Similarly, in the Rec assay, which gives an indication

of repairable DNA damage, 2,4-D was not more toxic to the DNA repair-deficient strain of Bacillus subtilis M45 (Rec^-) than to strain H17 (Rec^+), suggesting that the compound does not damage DNA (Shirasu 1975 and Shirasu et al. 1976). However, in subsequent tests by Simmon (1979), 2,4-D was relatively more toxic to the DNA repair-deficient strain, but only at the highest concentration tested (5 mg/disc; concentrations ranged from 0.01 to 5 mg/disc).

In the yeast, Saccharomyces cerevisiae, strain D4 gene conversion was increased by concentrations of 2,4-D above 400 ppm. Mitotic recombination in S. cerevisiae strain D5 was also increased by 2,4-D at 300 ppm (Siebert and Lemperle 1974; Zetterberg et al. 1977; cited in International Agency for Research on Cancer 1977). S. cerevisiae RAD18 (a histidine-dependent haploid strain) was reverted to histidine independence at levels of 150 ppm 2,4-D and above (Zetterberg 1978). Both mutagenic and toxic effects in S. cerevisiae were dependent on low pH (4.5 and 4.3 tested). In S. typhimurium, although toxicity to the bacteria was pH dependent, 2,4-D was nonmutagenic in strains TA1535 and TA1538 even when tested at concentrations of 30 to 80 ppm at pH 4.3 (Zetterberg et al. 1978).

2,4-D did not induce forward mutations in bacteriophage to E. coli or revert E. coli bacteriophage mutants to the wild T_4 phenotype (Anderson et al. 1972). Das and Singh (1978) observed no increase in morphological mutants when clonal populations of several blue-green algae were treated with 100 to 1,000 ppm 2,4-D.

In host-mediated assays with S. cerevisiae strain D4 or with S. typhimurium strains TA1530 or TA1531, as indicator cells, no mutagenic effects were observed when adult mice were given 6 mg 2,4-D (200 mg/kg body weight) by gavage and microorganisms recovered from the peritoneal cavity were examined (Zetterberg et al. 1977). Serum from rats treated orally with 2,4-D was not mutagenic when tested in vitro with S. typhimurium (Styles 1973).

The feeding of adult male Drosophila melanogaster fruit flies 4.5 or 9.0 mM 2,4-D in sucrose did not increase the frequency of recessive lethals (Vogel and Chandler 1974). In another study (Magnusson et al. 1977), a slight increase in recessive lethals was reported when larvae were fed corn agar containing 1,000 ppm 2,4-D. Larvae of genetically stable and unstable strains of D. melanogaster were treated with 25 ppm 2,4-D. No mutagenic effect was seen with the stable strain. A significant increase ($p \leq 0.001$) was seen in the frequency of somatic mutations (pigmented sectors for eye color) in males of the unstable strain in which spontaneous mutations occur (Rasmuson and Svahlin 1978).

Embryonic bovine kidney cells (EBK) and bovine peripheral cells (BPB) were exposed in vitro to concentrations of 1 to 1,000 ppm 2,4-D for 6 to 9 hours resulting in the stimulation of mitosis. No chromosomal aberrations were detected in the PBB cells, but nucleolar abnormalities and polyploid mitotic stages were observed in the EBK cells (Bongso and Basrur 1973).

Fahrig (1974) reported that human lymphocytes exposed in vitro to 2,4-D showed no increase in chromosomal aberrations. In contrast, an increase in chromosomal aberrations was observed at all but the lowest concentrations to which human lymphocytes were exposed (range of concentrations was 0.002 to 50 ppm) in another study (Pilinskaya et al. 1974 in Hayes 1982). Korte and Jalal (1982) exposed cultures of human lymphocytes to concentrations of 2,4-D ranging from 0.2 to 60 ppm for 20 hours. Although statistically insignificant increases in chromosome aberrations (deletions and gaps) were observed at low concentrations, significant increases occurred at concentrations of 50 and 60 ppm, indicating that at these or higher levels, 2,4-D may be a potential mutagen to human cells in culture.

In mice, toxic concentrations (100 to 300 mg/kg body weight) administered orally as a single dose significantly increased the frequency of aberrant metaphases two to four-fold. Single fragments were the primary aberration observed (Pilinskaya et al. 1974 in International Agency for Research on Cancer 1977). The oral administration of 2,4-D to rats did not induce chromosomal aberrations in their bone marrow cells (Styles 1973).

The intraperitoneal administration of 100 mg 2,4-D per kg body weight to mice did not increase the number of micronuclei in the erythrocytes of the bone marrow (Jenssen and Renberg 1976).

In an assay for detecting unscheduled DNA synthesis (UDS) human embryonic lung cells of the WI-38 strain were exposed to 2,4-D at concentrations of 0.1 to 100 ppm in the presence and absence of a metabolic activation system. There was no evidence for the induction of UDS, indicating that 2,4-D did not interact with DNA and that no DNA damage-repair took place in these cells (Simmon 1979). Similarly, cultured rat hepatocytes showed no evidence of UDS after exposure to 2,4-D (Probst and Hill 1981 and Probst et al. 1981). Earlier, Balo-Banga et al. (1973) showed that DNA-repair synthesis induced by either gamma rays or ultraviolet light was inhibited when irradiated cells were subsequently treated with 10^{-4} and 5×10^{-4}M 2,4-D.

When given as a single intraperitoneal injection of 125 mg 2,4-D per kg body weight, or when given orally on 5 successive days

for a total dose of 75 mg/kg body weight, 2,4-D did not increase dominant lethal mutations in mice (Epstein et al. 1972).

Studies in higher plants (Seiler 1978), in most cases, show no mutagenic activity of 2,4-D. However, chromosomal anomalies have been observed in a number of cultivated plants and weeds (Mohandas and Grant 1972; Amer and Ali 1974; and Khalatkar and Bhargava 1982), as well as in plant cells cultured in vitro (Singh and Harvey 1975a,b and Ronchi et al. 1976). Some of these effects may be the result of general toxicity due to the herbicidal and auxin-like action of 2,4-D. Owing to the complex physiological activities of 2,4-D on plant cells, the significance for animals and man is not clear.

3.0 Environmental Fate

2,4-D and its formulations are effective systemic herbicides used to control broadleaf weeds in cereal crops, sugarcane, turf, and pastures as well as in noncrop situations (Weed Science Society of America 1983). Formulations of 2,4-D tend to be toxic to broadleaf nontarget plant species, with the highly volatile esters particularly prone to causing toxic effects in crops close to application sites if care is not taken. In addition to its herbicidal properties, 2,4-D acts as a plant growth regulator. At low application rates, it can be used to induce rooting and blossom set, to control ripening of bananas and citrus fruits, and to delay dropping of some fruits until harvest (Weed Science Society of America 1983). Persistence of 2,4-D is relatively short in most soils and is moderate in water (Mullison 1981; Ghassemi et al. 1981; Weed Science Society of America 1983; and Ashton 1982). It has a highly variable toxicity to fish and aquatic invertebrates depending upon formulation, environmental parameters, and species, and has a generally low to moderate toxicity to most other animal species.

Formulations of 2,4-D are absorbed by both plant roots and foliage and are translocated up and down the plant stem. The major sites for 2,4-D accumulation are in meristematic tissues. 2,4-D is readily metabolized in plants by a variety of metabolic pathways (Weed Science Society of America 1983 and Ghassemi et al. 1981). In animals, 2,4-D tends to be readily excreted in the urine and does not bioaccumulate (Hayes 1982).

3.1 Bioaccumulation and Metabolism

Bioaccumulation and metabolism of 2,4-D in animals, plants, and microorganisms are extensively reviewed in Loos (1975) and in Ghassemi et al. (1981). Mullison (1982) has reviewed the physiology of 2,4-D in plants, and a number of studies reviewed in Section 3.1.2, along with Hayes (1982), examine the bioaccumulation and metabolism of 2,4-D in animals.

3.1.1 Plants

Since its introduction as a herbicide in the mid-1940's, the physiology and toxicity of 2,4-D, along with other phenoxy herbicides, has been studied extensively. Loos (1975) has reviewed in detail the behavior of 2,4-D and other phenoxy herbicides in plants. Discussion of 2,4-D bioaccumulation and metabolism in this section will briefly consider uptake, translocation, mode of action, metabolism, persistence, and residues with examples drawn primarily from the recent literature.

Uptake of 2,4-D by plants occurs through leaves, stems, and roots. Of particular concern in foliar applications of herbicides, including 2,4-D, is the problem of penetrating the cuticular surface of the leaf. The cuticle is composed of waxes and cutin and is more rapidly penetrated by 2,4-D formulations with high lipid solubility, such as the ester formulations and the free-acid form of 2,4-D, than by salt formulations derived from strong bases, such as the potassium and sodium salts of 2,4-D. Absorption into green stems is similar to foliar absorption, but bark covering of stems is an added barrier to penetration by aqueous solutions. Basal sprays of 2,4-D, therefore, are usually mixed with an oil carrier, such as diesel oil, to enhance penetration. Aqueous solutions can, however, be applied directly to the sap-wood through a cut in the bark, as by a "hack-and-squirt" application. Root-applied 2,4-D formulations, including salt formulations, are readily absorbed by plants (Loos 1975). Mayeux and Scifres (1980) studied foliar uptake of 2,4-D and reported that leaves of Drummond's goldenweed absorbed 50 percent of available 2,4-D (dimethylamine) within 5 days of application at a rate equivalent to 2 lb/acre (2.2 kg/ha) in July and November. Detached leaves of goldenweed absorbed significantly more 2,4-D (potassium salt) 2, 4, and 6 hours after application when the carrier contained 0.5 percent (volume) of a commercial surfactant than when the carrier was pure water.

Translocation of 2,4-D, once absorbed in the plant, depends on a variety of factors including the point of entry into the plant, environmental conditions such as moisture and temperature, and the physiological status of the plant. In general, foliar-applied 2,4-D translocates readily and is carried with assimilated material (such as the material from photosynthesizing leaves) to growth sites in roots, shoots, flowers, and fruits. Foliar-applied 2,4-D in dark-grown plants is not translocated until sugar is available for transport in the phloem. Translocation is strongly affected by soil moisture and atmospheric humidity, with low soil moisture resulting in reduced translocation from leaves to roots and high atmospheric humidity stimulating downward translocation of 2,4-D. Increased temperature in the 68 to 86°F (20 to 30°C) range results in increased rates of translocation of 2,4-D. Translocation upward by root-applied 2,4-D is restricted and takes place primarily in the transpiration stream of xylem (Loos 1975). Studies with small aspen plants (Populus tremula, approximately 1 foot high), however, indicated that translocation of foliar-applied 2,4-D occurred mainly upward to the growing shoot tip. Very little additional herbicide ended up in the roots when injected via cut stems (Eliasson and Hallmen 1973). These data indicated a ready transfer of 2,4-D from phloem to xylem in the plant.

The herbicidal activity of 2,4-D is due to its interference with normal plant growth processes. It does so by acting like the normal plant growth hormones, called auxins. Normally in the plant, the concentration and distribution of auxins is precisely controlled by synthesis and degradation reactions, resulting in control of the growth and development of the plant. The absorption of 2,4-D disturbs this delicate balance by altering the normal distribution of growth-regulating substances.

In addition to acting physiologically like the natural auxins in plants, 2,4-D tends to persist in the plant tissues for a longer period than the natural hormone (Loos 1975 and Mullison 1982). Various physical manifestations of altered growth responses due to 2,4-D in plants have been observed, including: a downward-bending of plant parts due to more rapid growth of dorsal tissues; abnormal twisting and bending of stems; swelling and splitting of stems; altered leaf growth resulting in formation of fringes or altered patterns of vein formation; lack of proper expansion of young leaves; lack of normal root elongation; cessation of apical growth; and induction of secondary roots (Mullison 1982). Physiological effects of 2,4-D include inhibition of photosynthesis, RNA synthesis, protein synthesis, and lipid synthesis, as well as changes in carbohydrate metabolism and interference with various enzyme systems (Mullison 1982 and Ashton et al. 1977). Hanson and Slife (1969) proposed that the immediate cause of plant death due to auxin-like herbicides such as 2,4-D is dysfunction of leaf and root tissue due to abnormal growth, which in turn appears to be due to abnormal metabolism of nucleic acids.

Plants metabolize 2,4-D readily by a variety of pathways to various degradation products. These pathways and processes are discussed in detail in Loos (1975). Such metabolic pathways include degradation of side chains, metabolism of 2,4-D to unidentified compounds by lengthening of side chains in a manner similar to fatty acid synthesis, hydroxylation of the ring structure to form a hydroxyphenoxyacetic acid, formation of conjugates with normal plant constituents such as formation of a glucose ester of 2,4-D, cleavage of the 2,4-D ring structure, and formation of various unidentified metabolites. Studies by Mumma and Hamilton (1979 in Mullison 1982) indicate that 2,4-D resistant plants, such as corn, can convert 2,4-D into an inactive, and thus nontoxic, carbohydrate conjugate. In 2,4-D susceptible plants, however, 2,4-D is converted into amino acid conjugates which interfere with normal nucleic acid metabolism and protein synthesis. Based on studies with root-applied ^{14}C-labeled 2,4-D (sodium salt), Hallmen (1975) found that 2,4-D was converted into complexed forms in tolerant species, but very little complexing of 2,4-D resulted in intolerant species. Derivatives of 2,4-D, such as esters, appear not to function as plant growth regulators until

they are first converted to an active acid form (Loos 1975). This conversion occurs rapidly, with most esters converted to the acid form within approximately half an hour (Scifres 1977).

Several studies have addressed the persistence of 2,4-D residues following application to various types of vegetation in both laboratory and field situations. Table 3-1 summarizes some of these studies. In addition, Norris (1981) discusses the persistence of 2,4-D in forest vegetation following application. Residues of 2,4-D are generally relatively nonpersistent in plants.

3.1.2 Animals

Various reviews of animal metabolism and bioaccumulation of 2,4-D and its formulations include Mullison (1981), Loos (1975), Hayes (1982), Lommen (1980), and Minnesota Department of Health (1978). Appendix F summarizes the available literature on human metabolism and bioaccumulation of 2,4-D as well as the data on other animal species from the recent literature. Bioaccumulation of 2,4-D or its formulations is low and any material that is absorbed tends to be rapidly eliminated, primarily via the kidney. For the most part, 2,4-D is not metabolized in animals, although the ester formulations are usually hydrolyzed to an acid form. Some studies have indicated the formation and excretion of metabolites of 2,4-D in the form of taurine conjugates in fish, or of other unidentified conjugates.

In general, 2,4-D and its various formulations, except nonpolar esters, are more soluble in water than they are in organic solvents or lipids. As a consequence of this differential solubility, 2,4-D does not tend to accumulate in the bodies of animals, but rather is carried in blood and interstitial fluid which comprise the aqueous portions of the body, and in the gut and kidney during ingestion and excretion. Although animals that are exposed to 2,4-D will take up some of the chemical, the bioaccumulation ratios (amount in tissue compared to ambient levels) will be low and the small amounts accumulated will be rapidly eliminated once exposure ceases (Norris 1981). Bluegills exposed to 3 ppm butoxyethanol ester of 2,4-D for 8 days had a mean body concentration of < 0.05 ppm (Sigmon 1979b), while the same species exposed to 2 ppm dimethylamine salt of 2,4-D for 7 days reached a whole body concentration 0.98 ppm (Sikka et al. 1977). Aquatic animals dosed with 2,4-D or its formulations by injection rapidly eliminated the injected material. Bluegills injected intraperitoneally with radiolabelled dimethylamine salt of 2,4-D excreted 90 percent of the ^{14}C-labeled 2,4-D within 6 hours (Sikka et al. 1977). Winter flounder, similarly injected with radiolabelled 2,4-D acid, excreted almost 53 percent of the labeled material within 6 hours, 10 percent of which was a conjugate of 2,4-D and 90 percent of which was unchanged 2,4-D (Pritchard and James 1979). Dogfish sharks, also injected with 2,4-D, rapidly excreted

Table 3-1

Summary of Residue and Persistence Studies with 2,4-D

Formulation	Application Data	Plant	Residue Levels and Time After Application		References
			ppm Residue	Days	
Polyethylene glycol butyl ether ester of 2,4-D	3 lb a.e. per acre in water. Simulated aerial application in May. South California	Chamise	95 70 69 20 16 3.8	0 14 29 69 146 379	Plumb et al. 1977
Butoxy propyl ester of 2,4-D	4 lb a.e. per acre in water. Ground sprayer in April and May. South California	Chamise	221 60 35 22 12 0.1	0 30 60 90 180 360	Radosevich and Winterlin 1977
Butoxy propyl ester of 2,4-D	4 lb a.e. per acre in water. Ground sprayer in April and May. South California	Grass and Forbs	269 21	0 30	Radosevich and Winterlin 1977
2,4-D amine	1 lb a.e. per acre in water. Simulated aerial application in June.	Grass	80 70 45 30 6 1	0 7 14 28 56 112	Morton et al. 1967
Butoxyethyl ester of 2,4-D	Rate not given, in diesel oil. Glass house application. Sweden	Poplar	2300 2500 1800 1300 870	1 3 9 37 365	Eliasson 1973
"Ester" of 2,4-D	Ground. 0.22 lb per acre 0.67 lb per acre 2.0 lb per acre Sweden	Cowberry Bilberry Cowberry Bilbery Cowberry Bilberry	0.3 0.1 1.0 1.3 3.7 4.8	34 29 35 29 35 29	Raatikainen et al. 1979
2,4-D (formulation not specified)	Ground. 0.22 lb per acre 0.67 lb per acre 2.0 lb per acre Sweden	Bilberry	none detected (<0.05)	1 year	Siltanen et al. 1981

Source: Adapted with additional data from Norris 1981.

the injected material, eliminating nearly 60 percent of the dose within 24 hours. In the dogfish, however, 90 to 95 percent of the excreted material was the taurine conjugate of 2,4-D rather than the acid form (James and Bend 1976 and Guarino et al. 1977, both in Pritchard and James 1979). When 2,4-D was injected into the spiny lobster, the half-life of 2,4-D in the lobster was 6 to 8 hours (Tuey and James 1980 and James 1979).

Bioaccumulation studies of 2,4-D and its formulations have been conducted in mammals, including humans, rodents, and domestic livestock. Human studies have been based primarily on cases of accidental or intentional injestion of 2,4-D or its formulations, or on studies of 2,4-D uptake during application of 2,4-D. Ingested 2,4-D was eliminated in the urine by human subjects, with a half-life of 17.7 hours (Sauerhoff et al. 1976) and with 75 percent of the total dose excreted within 96 hours (Kohli et al. 1974) during controlled laboratory studies. In the first study (Sauerhoff et al. 1976), 12.8 percent of the excreted material was an unidentified conjugate of 2,4-D, while no metabolites were detected in the latter study (Kohli et al. 1974). Based on urinalyses of aerial application crews applying the propylene glycol butyl ether ester of 2,4-D in a forest situation at a rate of 2 lb a.e./acre and under controlled conditions, Lavy et al. (1982) determined that total doses ranged from nondetectable to 0.0557 mg/kg body weight for normal spray procedures. For crews using protective gear and special precautions, the levels ranged from nondetectable to 0.0237 mg/kg body weight. In general, the highest doses were observed in those individuals working most closely with the spray concentrate or handling spray equipment such as the pilots, mechanics, and batchman-loaders, as compared to supervisors and observers.

Lack of bioaccumulation and rapid urinary excretion of 2,4-D has been confirmed in several nonhuman mammalian studies. Khanna and Fang (1966 in Loos 1975 and in Hayes 1982) report 40 to 60 percent elimination of 60 to 100 mg doses of 2,4-D within 24 hours in a rat. In this study, dose appeared to influence excretion, with a more rapid proportional elimination of herbicide at the lower doses than at the higher doses. Rates of blood plasma clearance were greatest for butyl esters, followed by the isooctyl ester and the 2,4-D acid in mice given subcutaneous injections at a rate of 100 mg/kg body weight (Zielinski and Fishbein 1967 in International Agency for Research on Cancer 1977).

Finally, various studies have shown that residues of 2,4-D in humans and animals are low following exposure to 2,4-D and are virtually nonexistent in terms of background levels. Following treatment of irrigation canal banks with 4 lb a.e./acre of the dodecyl-tetradecyl amine and dimethylamine salts of 2,4-D, Schultz

and Whitney (1974) found only 0.30 and 0.675 ppm 2,4-D in breast muscle and liver tissue, respectively, 1 day later in the Common Florida gallinule. No detectable residues were found 4 days after spray. In the same study, these authors report that of 60 fish samples taken along the canal, 3 had residue levels greater than 0.01 ppm, 16 had detectable residue levels less than 0.01 ppm, and the rest had no detectable residue levels. Cows and sheep, fed up to 2,000 ppm 2,4-D in their diet for 28 days, had average residue levels of less than 1.0 ppm in muscle, fat, and liver (Clark et al. 1975 in Lommen 1980). The higher residues detected in kidney tissue in this study were interpreted as a reflection of the excretion of 2,4-D by the kidney. Milk samples from cows allowed to graze on pastures sprayed with 2,4-D (unspecified esters sprayed at an unspecified rate) contained 2,4-D residues of 0.01 to 0.09 ppm during the first 2 days after spray with lower levels thereafter (Klingman et al. 1966 in Minnesota Department of Health 1978). Of 416 samples of human urine from the general population, no samples tested positive for 2,4-D, with a few samples showing unquantifiable trace amounts (Kutz et al. 1978).

3.2 Soils

The fate and transport of 2,4-D in soil has been reviewed by several authors including Ghassemi et al. (1981) and Norris (1981). Loos (1975) discusses in detail the effect of soil microorganisms on 2,4-D.

2,4-D has a very low persistence (one month or less) in most soils compared to other herbicides, providing only temporary herbicidal effects (Ashton 1982). The acid and salt forms of 2,4-D are highly water soluble, while the ester forms tend to be water insoluble (Weed Science Society of America 1983). In general, 2,4-D is relatively mobile in soil compared with other herbicides (Ghassemi et al. 1981), although this mobility is strongly influenced by various factors such as the water solubility of the specific formulation and the organic content and pH of the soil. Microbial activity is the major means for degradation of 2,4-D in soil, with the most active breakdown occurring under conditions of warm moisture and high organic content. Volatilization of 2,4-D depends upon a number of factors including specific vapor pressure of the formulation and specific field conditions. Most formulations used in forestry applications are based on low-volatility forms of 2,4-D. Photodegradation does not appear to play any significant role in the loss of herbicidal activity in soil (Weed Science Society of America 1983; Ghassemi et al. 1981; and Norris et al. 1983).

The persistence of 2,4-D has been studied in a wide variety of soil types and under a wide range of environmental and laboratory conditions. Persistence of 2,4-D in most soils is short and is

generally less than one month (Ashton 1982). Table 3-2 summarizes selected studies on the persistence of 2,4-D in acid, salt, and ester formulations under laboratory and field conditions, primarily in North America and Europe, and primarily within the past decade. These studies confirm that 2,4-D and its formulations have a short persistence in most soil environments and that the primary mechanism for degradation of 2,4-D in the soil is microbiological activity. A review of the persistence of 2,4-D and its formulations in forest floor litter and in soil, particularly forest soils, is contained in Norris (1981). Of particular significance to forest applications of 2,4-D are that: (1) there are statistically significant (but ecologically unimportant) differences in persistence of 2,4-D in different forest floor materials; (2) purified forms of 2,4-D are more readily degraded than commercial formulations, probably due to synergistic effects of components of the formulation; (3) the half-life of 2,4-D formulations in forest floor material is independent of the initial applied concentration; (4) the persistence of 2,4-D in forest floor material is not significantly altered when the material has been pretreated with insecticides such as DDT or phosphamidon (a small reduction in 2,4-D degradation in material treated with carbaryl is not thought to be important in field situations); (5) in the case of 2,4-D esters, the rate of ester hydrolysis to the acid form is important and this rate decreases with decreasing soil moisture and with an increase in the molecular weight of the alcohol moiety of the ester; and (6) with 2,4-D in general, the rate of degradation decreases with decreasing soil temperature, decreasing soil moisture (although it is also slower in flooded soil than soil at 85 percent field capacity), and decrease in pH below 5.5 (Norris 1981; Smith and Hayden 1981 and Torstensson 1975).

Helling (1971) compared 2,4-D to 39 other herbicides in the laboratory and demonstrated that 2,4-D was mobile in a silty clay loam soil, although 2,4-D metabolites were less mobile than the parent compound. Studies with various formulations of 2,4-D indicate that it is not strongly adsorbed to soil (Davidson et al. 1980; Rao and Davidson 1979 and Scott and Phillips 1972) although it is rapidly and reversably adsorbed to organic material such as one would find in forest soils (Norris 1970, 1981 and Norris et al. 1983). In a laboratory study with ^{14}C-labeled 2,4-D in a silty clay loam soil, Scott and Phillips (1972) report that 2,4-D was the least adsorbable of 10 herbicides studied. Davidson et al. (1980) and Rao and Davidson (1979) examined movement of 2,4-D amine solutions of 50 and 5,000 ppm concentration through soil columns (silty clay loam, sandy loam, and fine sand) and report a higher mobility at the higher concentration. Percentage adsorption of 2,4-D is increased by higher levels of soil organic matter, greater amounts of exchangable aluminum, and decreasing soil pH (Moreale and van Bladel 1980). Although the Weed Science Society of America (1983) reports that

Table 3-2

Summary of Soil Persistence Studies with 2,4-D Formulations

Formulation and Application Rate	Effects	Comments	References
2,4-D (granular) applied at 1.16 lb/acre (1.3 kg/ha) to bluegrass turf in laboratory chambers in July (Maryland)	Residue concentration half-life in soil was 10 days.	--[a]	Nash and Beall 1980
2,4-D applied at rates of 0, 1, and 4 lb a.i./acre (0, 0.9 and 3.6 kg/ha) with fertilizer to barley and rapeseed plots on silt loam and sand in spring (Interior Alaska)	In fall, traces of 2,4-D found in 5 of 135 samples (all 5 from 1 silt loam and from sand). Highest concentration was 0.163 ppm 2,4-D.	No signs of leaching downward of herbicides. 2,4-D was applied to soil and leaf surfaces.	Knight and Lewis 1981
2,4-D incorporated into soil sample to give 10 and 1,000 ppm. Soil was sandy clay and was incubated in laboratory for 96 weeks. (Finland)	Within 6 weeks, 2,4-D concentration less than half initial concentration for field capacity moisture soil. Flooded soil at 63.4 to 66.0% of initial concentration at 24 weeks, <50% at 48 weeks, showing effect of soil moisture.	Soils kept at both field capacity moisture and water under flooding conditions for both herbicide concentrations.	Sattar and Paasivirta 1980
2,4-D (^{14}C-labeled) incorporated into laboratory soil samples (heavy clay) to give 2 ppm and incubated at various controlled temperatures and moistures. (Saskatchewan, Canada)	Rapid degradation of 2,4-D with half-life of about 7 days at 59 to 77°F (15 to 25°C) and soil moisture above 65% of field capacity. At lower temperatures and soil moisture, breakdown was also slower.	--	Smith and Hayden 1981
2,4-D (95%) applied in laboratory to soil, sand, silt, clay or organo-mineral complex to give 20 ppm a.i. by weight. (United Kingdom)	Half-life of 2,4-D was 10 days in nonsterile soil (8 day-lag followed by rapid decline) but breakdown in irradiated and autoclaved soil was inhibited.	Indicative that soil breakdown of 2,4-D is essentially a microbial process. Soil fractions (sand, silt, and clay) with microbial populations retained ability to degrade 2,4-D but at a slower rate than parent soil. No breakdown in clay which was sterile.	Burns and Gibson 1980

Table 3-2 (Continued)

Formulation and Application Rate	Effects	Comments	References
2,4-D (analytical grade, ^{14}C-labeled) applied to representative soils (sand and loam) at 10 ppm and incubated in the laboratory. (Belgium)	After 1 month, 80 to 95% of applied 2,4-D was degraded.	Degradation curves typical of microbial adaptation and growth. Soils with pH < 6.0 with a low degradation rate.	Moreale and van Bladel 1980
2,4-D (analytical grade and liquid commercial formulation at 4 lb/gallon) incorporated into a calcareous loam at a rate equivalent to 5 tons/acre (11,227 kg/ha) and incubated in laboratory. (Mississippi)	Forty-five percent relative inhibition in CO_2 production with 2,4-D (analytical grade) compared to control. Formulated 2,4-D resulted in more CO_2 production (approximately 30%) than controls.	Both analytical and commercial 2,4-D resulted in lower bacterial populations compared to controls. Streptomyces enchanced with analytical 2,4-D and inhibited with commercial; fungi enchanced with analytical, unaffected by commercial.	Stojanovic et al. 1972
2,4-D acid applied at 2 lb/acre (2.2 kg/ha) to small plots of clay, sandy loam, and silty loam, soils in May. (Saskatchewan, Canada)	No 2,4-D detected in the 0 to 4 inch (0 to 10 cm) level after 22 weeks.	No field leaching detected.	Smith and Hayden 1976 in Smith 1982
2,4-D (unspecified formulations). (U.S.A.)	One hundred eighty-eight samples from cropland soils were analayzed for 2,4-D residues; 3 samples were positive, with a mean level of <0.01 ppm.	Only samples from soils where 2,4-D had been used were analyzed.	Wiersma et al. 1972
2,4-D (unspecified formulations) applied at 839 lb/acre (940 kg/ha) with other herbicides in 1964-65. (Thailand)	Two of 6 samples in 1971 contained 2,4-D residues equivalent to 0.16 and 0.188 lb/acre (0.18 and 0.21 kg/ha).	Area used to calibrate aerial spray equipment.	Committee on Effects of Herbicides in Vietnam 1974 in International Agency for Research on Cancer 1977
2,4-D (acid) applied at 264.5 lb/acre to Florida site.	No 2,4-D detected 10 years later.	Area used to calibrate aerial spray equipment.	Young et al. 1975.
2,4-D incorporated in laboratory samples of heavy clay, clay loam, and sandy loam to give a concentration of 2 ppm. (Saskatchewan, Canada)	For soils kept at 85% of field capacity, the half-life for 2,4-D was less than 7 days. However, small amounts of 2,4-D were lost after 14 days at 15% of field capacity.	Soils maintained in the dark at 68 ± 1.8°F (20 ± 1°C) and 85% or 15% of field capacity (moisture).	Smith 1978

Table 3-2 (Continued)

Formulation and Application Rate	Effects	Comments	References
2,4-D incorporated into soil pits at 6, 16, and 35 inch (15, 40, and 90-cm) depths in silty clay loam and sandy loam in early summer. (Nebraska)	Degradation of 2,4-D was rapid under aerobic conditions, with essentially total dissipation within 5 months. Under anaerobic conditions, degradation was similar to aerobic condition in silty clay loam, but there were significant levels of phytotoxic activity after 41 months in all 3 levels in the sandy loam.	Degradation determined by soybean bioassay.	Lavy et al. 1973
2,4-D (^{14}C-labeled) incorporated in a sandy soil and in a sandy loam soil, at 2 ppm in pots and kept in a glass hous either with or without corn (maize) plants. (Germany)	During a 78-day period, 53 to 77% of the applied radioactivity was released as CO_2. Differences due to differing pH, clay content, water content, texture, and carbon and nitrogen, all influencing microbial activity.	Plant roots did not affect rate of mineralization of 2,4-D, but they did enhance formation of non-extractable residues.	Siebert et al. 1982
Dimethylamine salt of 2,4-D applied to small field plot at 2.0 lb/acre (2.2 kg/ha) to sandy loam, heavy clay and silty clay in northern prairie (Saskatchewan, Canada) in October and in all soils in October and in May.	No residues of 2,4-D were detected in the top 2 inches (5 cm) of soil in May following October applications or in October following May applications. Negligible residues (<2% of applied 2,4-D) found in 2 to 4 inch levels (5 to 10 cm) in October and in May.	--	Smith 1975
Diethanolamine salt of 2,4-D applied to soils from forest and grassland to give concentration of 4.79 ppm and incubated in laboratory. (Oklahoma)	Rapid degradation, without lag phase, with a 4 to 5 day half-life.	--	Altom and Stritzke 1973
Mixed amines of 2,4-D (Chipman 2,4-D Amine 80) applied to silt loam soil at 5, 10, and 20 lb a.e./acre (5.6, 11.2, and 22.4 kg a.e./ha) in late summer under field conditions. (Nova Scotia, Canada)	Maximum soil residues found 14 days after application with subsequent rapid decomposition. Less than 5% remained at 70 days. Small amounts penetrated to the 4 to 8 inch depth, but none found in the 8 to 12 inch depth.	Soil with 2.6% organic matter, pH 5.8, and cation exchange capacity of 12.0 meg/100g. Slight field leaching occurred.	Stewart and Gaul 1977

Table 3-2 (Concluded)

Formulation and Application Rate	Effects	Comments	References
Triethanolamine salts of 2,4-D, (^{14}C-labeled) applied to forest-floor at 2 lb a.e./acre (2.24 kg/ha) (presumably in the laboratory).	At 315 hours, 89% of the ^{14}C material had been released as CO_2.	--	Norris 1966 in Norris 1981
2,4-D ester and amine formulations applied to clay annually in spring since 1947 at rates of (0.5 and 1.69 kg/ha) to 1969 and 0.38 and 1.0 lb/acre (0.42 and 1.12 kg/ha) since 1969. (Saskatchewan, Canada)	Sampled every October since 1978 with samples from 0 to 4 inch (0 to 10 cm) depth. No detectable trace of 2,4-D (<0.05 ppm) could be found in any soil sample.	Indicative of no 2,4-D carryover for the last 34 years.	Smith and Hayden 1981
Butyl ester of 2,4-D applied to unspecified soil type in a field study at 0.375 lb/acre (0.42 kg/ha) in June. (Alberta, Canada)	No 2,4-D detected in the 0 to 2 inch (0 to 5 cm) level after 4 weeks.	--	Cochrane and Russell 1975 in Smith 1982
2,4-D (^{14}C-labeled) acid applied in the laboratory to soils at 1 ppm and 25°C.	Averaged of 80% loss in 4 days; average of 90% loss after 11 days.	No correlation of degradation rate with soil properties "Good" soil moisture.	McCall et al. 1981

[a]Data not available or not applicable.

salts of 2,4-D are leached in sandy soils, a wide variety of field studies indicate that 2,4-D does not leach downward through the soil to any appreciable extent. Stewart and Gaul (1977 in Norris 1981) were unable to find 2,4-D residues deeper than 8 inches (20 cm) 55 weeks after application. Despite its relative mobility as reported by Helling (1971), 2,4-D is not thought to leach into streams (Norris 1981) since it is adsorbed to soil organic material and rapidly degraded by soil microorganisms.

Microbial degradation is the major mechanism by which 2,4-D is lost from soil. Behavior of microbial populations treated with 2,4-D and the mechanisms by which 2,4-D is degraded by microorganisms are extensively reviewed by Loos (1975). Early evidence that microorganisms play an important role in the degradation of 2,4-D in soil came from stimulation of 2,4-D degradation under warm moist conditions with high soil organic matter, which are conditions that also stimulate growth of microorganisms. Other evidence came from correlations between 2,4-D degradation rates and aerobic bacterial populations in soil and from inhibition of degradation in dried or sterilized soils (Loos 1975). In soils that have not been treated with 2,4-D, microorganisms capable of degrading 2,4-D appear to exist at relatively low density [1 to 245 organisms per gram of soil in 8 soils from Natal in South Africa, Loos et al. (1979)]. Burns and Gibson (1980) demonstrated a lag in the degradation of 2,4-D in soil which they attributed to microbial enrichment of the soil. This lag was followed by a rapid decline of 2,4-D residues due to microbial activity. Moubasher et al. (1981) reported a stimulatory effect on total counts of fungi as well as on specific species of fungi when incorporated into soil at 1.9 to 15.2 ppm (dry soil). When incorporated into agar medium at 6.3 to 50.4 ppm, 2,4-D stimulated counts only of some species of fungi and was toxic to most species at medium and high doses.

Only minor losses of 2,4-D activity occur due to photodegradation and, for most formulations, due to volatilization (Weed Science Society of America 1983). The differences in volatility of 2,4-D formulations have been discussed in Section 1.2 and are illustrated in Table 1-3. Ester formulations of 2,4-D hydrolyze rapidly in soil to the acid form under suitably moist conditions. This hydrolysis appears to occur, at least in some instances, as a nonbiological reaction (Ghassemi et al. 1981). Khare and Dubey (1979) exposed 250 ppm solutions of 2,4-D continuously for 4 days to "light" and "far red" treatments (wavelengths not specified) and compared germination of weed seeds treated with light exposed 2,4-D and controls. They reported a significantly greater proportion of seeds germinating when exposed to "light" and "far red" treated 2,4-D compared to controls, indicating reduction of herbicidal activity due to light. In this study, controls consisted of exposure to herbicides held in

the dark, or to untreated herbicide. However, no attempt was reported to determine the rate of seed germination absent from any 2,4-D at all.

3.3 Water

Herbicides such as 2,4-D can enter ponds and streams by direct application or accidental drift; by inflow of herbicide previously deposited in dry streambeds, pond bottoms, or irrigation channels; by overland flow during periods of heavy precipitation; or by leaching from soils (Norris 1981). Norris and Moore (1970) have reviewed the processes by which forest applied herbicides are carried into streams and the fate these herbicides in aquatic systems. In a study with 2,4-D applied with either picloram or silvex for brush control on hill pastures in southern Oregon, Norris et al. (1982) reported that during a 7 month period following application, 4.5 grams of 2,4-D (representing 0.014 percent of the total applied amount) were discharged into streams. They further concluded that most of the herbicide discharged into streams in this study resulted from material deposited in dry stream channels or from stream banks. The herbicide was then carried in flow from fall rains, and did not result from overland transport of herbicides from upslope areas. Frank and Sirons (1980) reported an analysis of 949 samples from 11 agricultural water sheds in Ontario, Canada, finding that 66 samples (7 percent) contained 2,4-D residues ranging from 0.1 ppb to 320 ppb. The two highest residues, 320 ppb and 15.9 ppb, involved sample collections during application of 2,4-D to ditch and stream bank and in the latter case, involved spillage of 2,4-D material. Transport of 2,4-D to waters during 1975 to 1976 ranged from 0 to 0.0005 lbs/acre/year (0 to 553 mg/ha/yr), and during the 1976 to 1977 season ranged from 0 to 0.0001 lbs/acre/year (0 to 124 mg/ha/yr). The higher losses were due to ditch bank spraying, storm runoff from treated agricultural fields, and spillage. Transport losses from forest soils to water bodies should be considerably less than from agricultural soils, due to factors such as reduced surface runoff, adsorption to litter, absorption by plants, and possible greater organic material and microbiological activity in forest soils.

In aquatic environments, 2,4-D is usually ionized to the free anion, since salt formulations dissociate to the anion, and ester formulations rapidly hydrolyze to the anion (Halter 1980 in Ghassemi et al. 1981). For this reason, the fate and persistence of the free anion is of primary concern in aquatic environments. Various studies have shown that 2,4-D is readily degraded by microorganisms if environmental conditions are favorable for their growth; that 2,4-D esters are readily hydrolyzed in aquatic environments by nonenzymatic means; that although 2,4-D does photodecompose near the surface of water, photodecomposition is not a major mechanism for the loss of

2,4-D in waters; that 2,4-D esters can be lost from water by vaporization, especially at lower pH values; that 2,4-D does not adsorb readily to particles and sediment in water; and that maximum residues of 2,4-D in aquatic environments, when they are found, are in the parts per billion (ppb) range rather than in parts per million (ppm) (Ghassemi et al. 1981). The behavior of 2,4-D and its formulations in aquatic environments has been reviewed by Ghassemi et al. (1981), Lorz et al. (1979), Norris (1981), Norris et al. (1983), and Scott et al. (1981).

In several studies, 2,4-D applied to fish culture ponds resulted in stimulation of microbial growth, growth of rooted plants, or growth of fish. Jana and De (1982) applied a commercial formulation of 2,4-D (specific formulation not given) at a dosage of 500 ppm to fish ponds. They reported increased populations of heterotrophic bacteria over a period of one year in these ponds compared to controls. This stimulation was attributed to an increase in bacterial nutrients as a result of death of the planktonic algae populations due to 2,4-D. Boyle (1980) treated fish ponds with the dimethylamine salt of 2,4-D at 4.5 or 8.9 lb a.i./acre (5.0 and 10 kg/ha) to give 2,4-D concentrations of 0.5 and 10 ppm herbicide in the water. At 0.5 ppm, growth of rooted macrophytes was stimulated, and, at 1.0 ppm, fish growth rates were accelerated. The increased growth rate of fish may be due to increased phytoplankton productivity, although the author did not rule out the possibility of a direct stimulatory effect of 2,4-D on fish growth.

Degradation of 2,4-D in aquatic systems will occur by microbial metabolism if environmental conditions are favorable in terms of pH, nutrient levels, and temperature so as to promote growth of microorganisms capable of degrading 2,4-D. However, such degradation may be slow in cool, nutrient poor conditions with dilute concentrations of 2,4-D, as may be found in most naturally occurring surface waters (Ghassemi et al. 1981). Zepp et al. (1975) reported that all 2,4-D esters hydrolyzed in water. Based on direct kinetic measurements made on methyl and butoxyethyl esters of 2,4-D in water, they calculated hydrolysis half-lives ranging from 0.6 days for 2-butoxyethyl ester to 37 days for 2-octyl ester at 28°C and at a pH of 9. Half-lives were considerable longer at a pH of 6. They also calculated photolysis and vaporization half-lives. At 25°C, they obtained photolysis half-lives in the 16 to 29 day range and half-lives for vaporization in the 1.1 to 895 day range (based on a one-meter deep water body) on a clear September day in the southern United States. In basic water, hydrolysis is the most important mechanism for degradation of esters, while in acidic and neutral waters, vaporization may be more important. Nesbitt and Watson (1980a,b) studied microbial degradation of 2,4-D in a river in southwest Australia and reported that the rate of degradation was

increased with increased nutrients, sediment load, and dissolved organic carbon. Khare and Dubey (1979) demonstrated that visible light and far red light both reduced phytotoxicity of 2,4-D in a laboratory study.

Despite often heavy application rates directly into water bodies or along ditch and canal banks for control of aquatic weeds, residues of 2,4-D in water bodies seldom exceed 10's of ppb. Table 3-3 summarizes selected residue studies of 2,4-D in water is and indicative of the low levels of 2,4-D that actually occur in water bodies following application.

Table 3-3

Summary of Selected Water Residue Studies with 2,4-D and its Formulations

Formulation and Application Rate	Residues	Comments	References
2,4-D applied to National Forest lands, Pacific Northwest Region, 1974 to 1978.	Two hundred fifty four samples representing 133 sprayings monitored for 2,4-D were analyzed. Of the 133 sprayings, 117 had no confirmable residues, 13 had residues <5 ppb, 2 had residues of 5 to 10 ppb, and 1 had a residue of 40 ppb.	--[a]	Neisess 1983
2,4-D applied in states west of the Mississippi.	Of 62 samples taken January to September, none had detectable levels of 2,4-D. For the two year period of October 1966 to September 1968, 36 samples contained detectable levels of 2,4-D, with a maximum concentration of 0.35 ppb.	--	Brown and Nishioka 1967 and Manigold and Schulze 1969
2,4-D applied in 11 agricultural watersheds in Ontario, Canada.	Of 949 samples, 66 contained 2,4-D; 58 with 0.1 to 1 ppb, 6 with 1 to 10 ppb, 1 sample with 15.9 ppb, and one with 320 ppb.	The high concentrations were associated with ditchbank spraying or spill.	Frank and Sirons 1980
Dodecyl-tetradecyl amine salts of 2,4-D applied to over 7,000 acres along perimeter canal in Loxahatchee National Wildlife Refuge, Florida. Applied at 4 lb a.e./acre (4.48 kg/ha) in June.	Water samples were taken at 3 sites up to 113 days after application. Highest residue levels, 1 day after treatment were 37 and 16 ppb, all other samples were less than 10 ppb. The highest residues in hydrosol were 5 ppb, occurring 3 to 15 days alter treatment.	Initial application was followed by spot treatments with same formulation or dimethylamine salts over a 4-month period.	Schultz and Whitney 1974

Table 3-3 (Continued)

Formulation and Application Rate	Residues	Comments	References
Dimethylamine salt of 2,4-D applied at 40 lb a.e./acre for submersed aquatic plant control (Site not given).	Residues of 1.8 ppm 8 hours after application.	As cited in Lorz et al. 1979, "residues (in water) greater than 0.02 ppm at only 2 of 19 stations 4 weeks after treatment."	Wojtalik et al. 1971 in Schultz and Whitney 1974
Dimethylamine salt of 2,4-D applied to irrigation canals at 4 lb a.e. and 8 lb a.e./acre (4.48 kg and 8.96 kg/ha) in Southern Louisiana	Highest concentrations of 15.8 and 10.4 ppb in samples at treatment site 48 and 0.5 hour after application. Highest average values 2 hours after application and were 4.9, 4.7 and 4.6 ppb 92, 806, and 403 meters downstream, respectively.	Low concentrations due to rapid dilution. Adsorption to suspended clay particles was minimal. If spray properly applied, it will cover vegetation and be absorbed or degraded before entering water.	Foret and Barry 1979
Dimethylamine salt of 2,4-D applied to 11 ponds in Georgia, Florida, and Missouri	Residues declined to 5 ppb in water samples taken 28 days after application in Florida and Georgia, and 56 days in Missouri. Highest sediment residues of 170 ppb in Missouri pond treated at 8 lb/acre, residues never more than 500 ppb in Florida/Georgia pond sediment.	--	Schultz 1974 in Foret and Barry 1979
Dimethylamine salt of 2,4-D applied at 4.5 and 8.9 lb/acre (5 and 10 kg/ha) to experimental fish ponds (Missouri)	Residues peaked 7 days after application at approximately 1.2 ppm and 0.6 ppm, and declined to below detectable limit (0.1 ppm) at 56 days.	—	Boyle 1980

Table 3-3 (Concluded)

Formulation and Application Rate	Residues	Comments	References
Triisopropanol amine salts of 2,4-D with picloram at 4.1 and 6.0 lb/acre in Southern Oregon June	Residues from area receiving 4.1 lb/acre were a maximum of 22 ppb during a storm event in October. Maximum 10 ppb occurred in September from the site receiving 6.0 lb/acre.	--	Norris et al. 1982
Butyl ether ester of 2,4-D (granular) applied to 1144.4 acres (463.3 ha) at 8.1 lb/acre (9.1 kg/ha) in June and to 512.8 acres (207.6 ha) at 16.5 lb/acre (18.5 kg/ha) in August 1978 in Reservoir in Eastern Oklahoma.	Of 240 samples tested for butyl ether ester, only 1 contained detectable residues (3 ppb) and it was taken 4 hours after application. A possible trace detected in a 2nd sample. Of 158 samples analysed for 2,4-dichlorophenol (breakdown product), none were positive.	State (Oklahoma) standard of 100 ppb was not exceeded.	Morris and Jarman 1981
Butyl ether ester of 2,4-D applied at 40 to 100 lb a.e./acre to reservoir in the Tennessee Valley Authority system.	Residues of 6.8 ppb found 8 hours after application.	--	Smith and Isom 1967 in Schultz and Whitney 1974
Low volatile esters of 2,4-D applied at 2 lb/acre in June to 2 watersheds in Eastern Oregon.	Maximum residues of 132 ppb 1.75 hours after treatment. Residues of 0 ppb by 168 hours.	Highest concentrations from watershed having streams flowing through it.	Norris 1967 in Norris and Moore 1970

[a]Data are not available or not applicable.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to 2,4-D formulations during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved 2,4-D. Calculations of exposure to 2,4-D in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to 2,4-D are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service 2,4-D Application

Available application data for 2,4-D use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to 2,4-D differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of 2,4-D in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

In general, 2,4-D is rapidly degraded in the environment and does not bioaccumulate in animals. Consequently, the potential for incidental exposure of humans and wildlife to 2,4-D is low, existing only for a very short period immediately after its application.

4.2 Exposure

Estimates of potential exposure to 2,4-D are based on aerial as well as ground application. The maximum aerial application rate for 2,4-D (see Table 1-1) is 7.8 lb a.e./acre for right-of-way maintenance, while the compound is applied from the ground (ground foliar) at rates as high as 6.0 lb a.e./acre (excluding granular, hand broadcast, and injection application). In aerial application

Table 4-1

USDA Forest Service Application Data for 2,4-D (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Low Volatile Ester (35)	Aerial	8.5	298	3.9	4.8	5.8	5.7	Timber (23), Range (8) Noxious Weeds (1) Wildlife (1) Right-of-Way (1) Firebreak (1)
Low Volatile Ester (84)	Ground Foliar	5.6	471	6.3	12.5	7.9	15.2	Timber (35), Range (24) Noxious Weeds (15) Wildlife (3) Research (1) Engineering (2) Recreation (2) Nursery (2)
Amine (19)	Ground Foliar	4.3	82	5.1	13.3	7.2	16.2	Range (9) Noxious Weeds (5) Wildlife (1) Recreation (1) Engineering (1) Nursery (1), Timber (1)
Amine (25)	Injection and Cut Stump (undiluted)	13.5	338	5.3	33.8	6.3	49.4	Timber (19) Wildlife (6)

[a]Grand Total = 1,189

Source: Gross 1983

and in ground foliar application, 2,4-D is applied as a liquid. The calculations in Table 4-2 that are based on aerial application (e.g., spray/observer dose, wildlife dermal exposures, and water concentrations), assume an application rate of 7.8 lb a.e./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose) assume an application rate of 6.0 lb a.e./acre. For those ground applications in which 2,4-D is applied as a granular form, as well as for hand broadcast and injection applications, 2,4-D exposure is assumed to be equal to, or less than, exposures calculated in this analysis. Other specific assumptions, based upon the toxicological and chemical characteristics of 2,4-D and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Lavy et al. (1982) have estimated 2,4-D doses as high as 0.0557 mg/kg body weight for aerial applicators treating forests with 2,4-D at 2 lb a.e./acre. These figures are based on urinalyses of workers using normal precautions and customary clothing. This figure, while lower, is in the same order of magnitude as the data used for the occupational doses for the other herbicides in this study. To be conservative, this analysis will utilize the dose estimates for mixer/loader, observer, and backpack sprayer discussed in Section 4.0 of the General Introduction to the Herbicide Background statement.

As indicated in the General Introduction, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for 2,4-D are based on exposures on a per pound per acre application rate multiplied by 7.8 lb/acre for observers and 6.0 lb/acre for backpack sprayers. In the case of the aerial spray observer, exposure estimates are based upon the maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. At 7.8 lb a.e./acre application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.312 mg/kg
(0.04 mg/kg at 1 lb/acre application rate)

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for 2,4-D

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg[b]		
Observer	up to 0.312 mg/kg		
Backpack Sprayer	0.24 mg/kg		
Environmental Exposure			
Fish	0.078 ppm (Immersion)[c]		
Rabbits	3.143 mg/kg	Negligible	8.19 mg/kg
Deer	0.788 mg/kg	Negligible	11.23 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.0016 mg/kg (2 days)
(Rabbits)	NA	NA	0.039 mg/kg (2 days)
(Deer)	NA	NA	0.047 mg/kg (2 days)
(Water)	NA	NA	0.0031 mg/kg (1 day)
(Berries)	NA	NA	0.0078 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0039 mg/kg (1 day)
Toxicity Summary	Acute LD_{50}'s for rabbit = 1,400 mg/kg	Human TLV = 10 mg/m^3; short-term TLV = 20 mg/m^3	Acute oral LD_{50} for mule deer is 400 to 800 mg/kg (acid). Acute oral LD_{50} for rabbit is 424 to 800 mg/kg.[f]
	Most studies, LC_{50} >1 ppm for fish (esters); >>1 for salts.		

[a]Assumes aerial application at 7.8 lb active ingredient per acre and ground foliar application at 6 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure and are on a daily basis.

[c]Fish exposure results from immersion in water containing 2,4-D.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

[f]Various formulations.

For ground applications at 6.0 lb/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.24 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by 2,4-D application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 7.8 lb a.e./acre aerial spray:

Rabbits in Aerial Spray Zone: 3.1434 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.7878 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure

orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. This exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of 2,4-D in water, calculated as:

Water concentration: 0.078 ppm (0.01 ppm at 1.0 lb/acre application rate)

This concentration of 2,4-D represents the estimated maximum concentration immediately after spraying. If heavy rainfall occurred immediately after spraying, higher transient concentrations could occur in streams and ponds. However, such high concentrations result from high application rates or result from precipitation soon after application, and would be transient since they are rapidly diluted by resident waters. Actual levels of 2,4-D residues in ponds and streams monitored after application of 2,4-D to surrounding watersheds were in the ppb-range in a variety of studies summarized in Table 3-3.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of 2,4-D by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is aerially sprayed at 7.8 lb a.e./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 11.232 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 8.190 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of 2,4-D in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.078 ppm

- bioconcentration: 1.0 (actual bioconcentration for 2,4-D is < 1.0 in most studies [see Appendix F]).

Fish tissue concentration: 0.078 ppm (0.078 mg/kg)

Deer and rabbit tissue concentrations include 2,4-D accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied 2,4-D is absorbed at a rate of 10 percent. It is also assumed that oral exposure of deer and rabbit to 2,4-D lasts only for 2 days and that only 10 percent of the ingested material is assimilated, since ingested 2,4-D is rapidly eliminated in animals (See Appendix F). Deer tissue concentrations of 2,4-D are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.7878 mg/kg (dermal exposure) x 0.1 = 0.07878 mg/kg

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 11.232 mg/kg/day (oral exposure) x 0.1 x 2 days = 2.2464 mg/kg

Total Deer Tissue concentration = 0.07878 mg/kg + 2.2464 mg/kg = 2.32518 mg/kg (2.325 ppm)

Rabbit tissue concentrations of 2,4-D are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 3.1434 mg/kg (dermal exposure) x 0.1 = 0.31434 mg/kg

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 8.190 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.6380 mg/kg

Total Rabbit Tissue Concentration = 0.31434 mg/kg + 1.6380 mg/kg = 1.95234 mg/kg (1.952 ppm)

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to 2,4-D from eating meat are derived:

Fish meat: 0.078 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.00156 mg/kg

Deer meat: 2.325 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0465 mg/kg

Rabbit meat: 1.952 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0390 mg/kg

Water and plant consumption by man would result in the following oral exposures to 2,4-D based on assumptions stated in the General Introduction to the Herbicide Background Statements at an aerial application rate of 7.8 lb a.i/acre:

Water: 0.00312 mg/kg (0.0004 mg/kg at 1 lb/acre application)

Berries: 0.0078 mg/kg (0.001 mg/kg at 1 lb/acre application)

Mushrooms: 0.0039 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of 2,4-D to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares exposure estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rabbit, mule deer, and human acute oral LD_{50}'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels anticipated for USDA Forest Service applications.

The International World Health Organization has established an acceptable daily intake of 0.3 mg/kg for 2,4-D by humans (Mullison 1981). The oral exposures (environmental) presented in Table 4-2 are below this value and represent exposures that would occur only on a transient basis.

Appendix A

Summary of 2,4-D Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D	Honeybees (Apis mellifera; Insecta)	Oral (in feed)	--[a]	At 1,000 ppm weight, brood production severely impaired; at 100 ppm, amount of brood reduced; at 10 ppm, no adverse effect on brood compared to controls.	Effects were temporary and abated when herbicide was removed.	Morton and Moffett 1972
2,4-D	Honeybees (Apis mellifera; Insecta)	Surface absorption (contact)	--	Amine salt formulations toxic only at "excessively high concentrations" while ester formulations very toxic (direct contact) at normal use concentrations.	No hazard to colonies fed 250 ppm a.i.	King 1964
2,4-D	Honeybees (Apis mellifera; Insecta)	Oral	--	LD_{50} = 0.105 mg	Classed as a stomach/contact poison with low toxicity to bees.	Glynne Jones and Connell 1954 in Way 1969
2,4-D	Honeybees (Apis mellifera; Insecta)	Oral	--	One hundred percent mortality in bees 4 days after feeding of 30 µg. Ten percent mortality at 3 days and 20% at 5 days and after feeding 20 µg.	--	Byrdy 1969 in Way 1969
2,4-D (purified)	Honeybees (Apis mellifera; Insecta)	Subchronic, oral	--	Newly emerged honeybees with half-lives of 33.4, 37.2, 40.4, 40.4, and 18.6 days with 0, 10, 100, and 1,000 ppm weight 2,4-D in feed, respectively.	Bees fed 10 and 100 ppm 2,4-D by weight in feed lived signifiantly longer than controls, bees fed 1,000 ppm by weight lived a significantly shorter lifespan.	Morton et al. 1972
2,4-D	Honeybees (Apis mellifera; Insecta)	--	--	LD_{50} = 11.525 µg/bee	--	Beran and Neururer 1955 in Pimentel 1971

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dimethyl-amine salt of 2,4-D (DMA-4 and Weedar 64®)	Honeybees (Apis mellifera; Insecta)	Surface absorption (contact)	up to 3 days	Mortality of bees sprayed with 2,4-D in water at a rate equivalent to 4 lb/acre was similar to that of bees sprayed with water alone.	--	Moffett et al. 1972
Dimethyl-amine salt of 2,4-D (Dow DMA-4)	Honeybees (Apis mellifera; Insecta)	Subchronic oral	--	Newly emerged bees had half-lives of 25.8, 44.4, 43.2, and 15.6 days with 0, 10, 100, and 1,000 ppm weight commercial 2,4-D salt in feed, respectively.	Half lives of bees fed 10 and 100 ppm were significantly longer than controls and bees fed 1,000 ppm. No difference between controls and bees fed 1,000 ppm.	Morton et al. 1972
Isooctyl ester of 2,4-D (Chipman 133, 2,4-D low volatile ester 4L)	Honeybees (Apis mellifera; Insecta)	Subchronic oral	--	Newly emerged bees had half-lives of 30.2, 27.2, 36.4, and 28.9 days with 0, 10, 100, and 1,000 ppm weight commercial 2,4-D ester in feed, respectively.	No significant difference in half-lives between controls and any treatment groups.	Morton et al. 1972
Butoxy-ethanol ester of 2,4-D (purified)	Honeybees (Apis mellifera; Insecta)	Subchronic oral	--	Newly emerged bees had half-lives of 39.0, 45.2, 48.2, and 15.6 days with 0, 10, 100, and 1,000 ppm weight 2,4-D ester in feed, respectively.	No significant difference in half-lives between controls and any treatment groups.	Morton et al. 1972
2,4-D (analytical grade)	Fruit flies (Drosophila melanogaster; Insecta)	Surface absorption	24 hours	Exposure to 40 μg 2,4-D per bioassay jar with 50 flies resulted in 1.7-fold increase in mortality with 2,4-D + DDT compared to insecticide exposure without 2,4-D.	Increase in mortality with DDT + 2,4-D significant at 95% level.	Lichtenstein et al. 1973

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (Chipman 2,4-D amine No. 4)	Planktonic dipteran (*Chaoborus punctipennis*; Insecta)	Surface absorption, static bioassay	24 hours	IC_{50} = 1,490 ppm (59°F) and 1,124 ppm (68°F)	Larvae (3rd and 4th instar) tested at 59°F (15°C) and 68°F	Bunting and Robertson 1975
			49 hours	IC_{50} = 1,142 ppm (0 to 1,871 ppm) at 59°F (90% limits)		
			96 hours	IC_{50} = 890 ppm (421 to 1,211 ppm) at 59°F (90% limits)		
Buyoxy-ethanol ester of 2,4-D	Midge (*Chironomus*, *sp.*; Insecta)	Surface absorption	--	Larvae and pupae exposed to 0, 1, and 3 ppm 2,4-D at 68°F, 77°F, and 86°F, respectively. Greater larval mortality occurred with 2,4-D than with controls. A reduced rate of pupal emergence occurred at 86°F in 1 and 3 ppm 2,4-D compared to lower temperatures on controls (0 ppm 2,4-D).	--	Sigmon 1979
Butoxy-ethanol ester of 2,4-D	Mosquito (*Anopheles quadrimaculatus*; Insecta, larvae)	Surface absorption	--	Larvae survived 100 ppm 2,4-D in laboratory. Adults from the treated larvae raised through two generations with no impairment of hardiness or reproductive ability.	--	Smith and Isom 1967 in Mullison 1970a,b
2,4-D (free acid)	Copepod (*Cyclops vernalis*; Crustacea)	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} = 37.42 ppm LC_{50} = 8.72 ppm 5.32 to 11.57 ppm) (90% limits)	0-4 hours nauplii, tested at 68°F (20°C). Median lethal times ranged from 110.12 (95.57 to 344.41) hours with 5 ppm 2,4-D to 36.11 (31.49 to 41.40) hours with 40 ppm 2,4-D.	Bunting and Robertson 1975; Robertson 1975; and Robertson and Bunting 1976

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 417.8 ppm	78°F, 20°C	Presing 1981
2,4-D (acid)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	70°F, 20°C	Sanders 1970
	Scud (Gammarus fasciatus Crustacea)			TL_{50} = 3.2 ppm	60°F, 15.5°C	
2,4-D	Cladoceran (Daphnia lumholtzi; Crustocea)	Surface absorption	up to 71 hours	At 10 ppm concentration, 50% died at 38 hours, 100% at 71 hours. At 20 ppm concentration, 50% died at 21 hours, 100% died at 31 hours.	—	George et al. 1982
2,4-D	Copepod (Mesocyclops leuckarti; Crustacea	Surface absorption	up to 30 days	No effect at concentrations of 1.0 to 50 ppm.	—	
2,4-D (acid)	Brown Shrimp (Panaeus aztecus; Crustacea)	Surface absorption	48 hours	Ten percent showed mortality or paralysis at 2.0 ppm 2,4-D.	—	Butler 1965
2,4-D (Technical, 98%)	Dungeness Crab (Cancer magister; Crustacea)	Surface absorption, static bioassay	96 hours	First instar zoeae LC_{50} and EC_{50} both >10 ppm. First instar juvenile crabs LC_{50} and EC_{50} both >100 ppm.	Tested at 55.4 ± 3.6°F (13 ± 2°C and 25 ± 0.5 pp thousand salinity. Egg hatching at 3.3 to 10 ppm was 80% or better, at 330 ppm was almost completely inhibited, with opaque unhatched eggs. For zoeael development, no effect at 33 ppm, inhibition at 100 ppm. Up to 33 ppm, first-stage zoeae with unaffected motility, but those at 100 ppm were mostly nonmotile if they survived.	Caldwell 1977 and Caldwell et al. 1979

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (Technical, 98%)	Dungeness Crab (Cancer magister; Crustacea)	Surface absorption, chronic exposure	69 days	Larval survival impaired by exposure to 10 ppm 2,4-D with only 50% survival to day 20 and less than 10% survival to day 50. Survival of larvae exposed to 1 ppm did not differ from controls. Juvenile crabs exposed to 10 ppm exhibited poorer survival after 10 days than controls or crabs exposed to 10 ppm in one experiment. In another, no effect was observed with 10 ppm or 1 ppm compared to controls. With adult crabs, no effect on survival was observed at concentrations of 10 ppm 2,4-D for a period of 85 days.	Exposure to 2,4-D at 1 and 10 ppm resulted in delayed molting during larval development. Juvenile molting advanced when crabs are exposed to 2,4-D at 10 ppm.	Caldwell 1977 and Caldwell et al. 1979
Alkanolamine salt of 2,4-D	Crayfish (Procambarus clarkii; (Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 1,389 ppm (1,174 to 1,681 ppm)	Immature individuals. Water temperature 68 ± 5.4°F (20 ± 3°C)	Cheah et al. 1980
Sodium salt of 2,4-D	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 1042.3 ppm	78°F, 20°C	Presing 1981
Dikonirt (71.2% sodium salt of 2,4-D and 1.8% dichlorophenol)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	24 hours 48 hours 96 hours	LC_{50} = 507.8 ppm LC_{50} = 372.1 ppm LC_{50} = 268.5 ppm	59°F, 15°C	

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Alkanolamine salt of 2,4-D	Copepod (*Cyclops vernalis*; Crustacea)	Surface absorption, static bioassay	48 hours	LC_{50} = 662.11 ppm (225.57 ppm a.e.) LC_{50} = 142.0 ppm (120.34 to 167.3 ppm) (54.8 ppm a.e. [46.45 to 64.6 ppm a.e.]) (90% confidence limits)	0-4 hour nauplii, tested at 68°F (20°C). Median lethal times ranged from 102.77 (92.36 to 124.80 hours with 100 ppm 2,4-D salt to 51.22 43.10 to 57.49) hours with 800 ppm 2,4-D salt.	Bunting and Robertson 1975, and Robertson and and Bunting 1976
Dimethylamine salt of 2,4-D (liquid, 49%)	Cladoceran (*Daphnia magna*; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 4.0 ppm (3.4 to 4.9 ppm)	Hard water (272 ppm $CaCO_3$). First instar, 70°F (21°C)	Johnson and Finley 1980, Sanders 1970
	Seed shrimp (*Cypridopsis vidua*; Craustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 8.0 ppm (5.9 to 10.8 ppm)	Hard water (272 ppm $CaCO_3$). Mature, 70°F (21°C)	
	Scud (*Gammarus fasciatus*; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	Hard water (272 ppm $CaCO_3$). Mature, 59°F (15°C)	Johnson and Finley 1980
	Glass shrimp (*Palaemonetes kadiakensis*; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 0.15 ppm (0.11	Mature, 70°F (21°C)	
Dimethylamine salt of 2,4-D	Scud (*Gammarus fasciatus*; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	60°F, 15.5°C	Sanders 1970
	Sowbug (*Asellus brevicaudus*, Isopodo; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	60°F, 15.5°C	
	Glass shrimp (*Palaemonetes kadiakensis*; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	70°F, 21°C	Sanders 1970
	Crayfish (*Oronectes nais* Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	60°F, 15.5°C	

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dimethyl-amine salt of 2,4-D	Brown shirmp (Panaeus aztecus; Crustacea)	Surface absorption	48 hours	Ten percent showed mortality or paralysis at 2.0 ppm.	—	Butler 1975
Butoxy-ethanol ester of 2,4-D	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption	--	Adults exposed to 0, 1, and 3 ppm 2,4-D at 68°F, 77°F, and 86°F had no statistically significant difference in oxygen consumption although mean respiration increased with temperature, and highest respiration at 3 ppm 2,4-D.	--	Sigmon 1979
Butoxy-ethanol ester of 2,4-D (Technical 62.5%)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	LC_{50} = 6.4 ppm (4.5 to 9.1 ppm)[b]	First instar, 70°F (21°C)	Johnson and Finley 1980
	Seed shrimp (Cypridopsis vidua; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 2.2 ppm (1.5 to 3.3 ppm)	Hard water (272 ppm $CaCO_3$). Mature, 70°F (21°C)	Johnson and Finley 1980
	Sowbug (Asellus brevicaudis; Isopod, Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 2.6 ppm (1.3 to 5.3 ppm)	Mature, 59°F (15°C)	Johnson and Finley 1980
	Scud (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 6.1 ppm (4.5 to 8.3 ppm)	Hard water (272 ppm $CaCO_3$). Mature, 58°F (15°C)	Johnson and Finley 1980
Butoxy-ethanol ester of 2,4-D	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 5.6 ppm	70°F, 21°C	Sanders 1970
	Seed shirmp (Cyridopsis vidua; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 1.8 ppm	70°F, 21°C	Sanders 1970
	Sowbug (Asellus brevicaudus; Isopoda, Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 3.6 ppm	60°F, 15.5°C	Sanders 1970

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Butoxy-ethanol ester of 2,4-D	Glass shrimp (Palaemonetes kadiakensis, Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 1.4 ppm	70°F, 21°C	Sanders 1970
	Crayfish (Oronectes nais Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} 100 ppm	60°F, 15.5°C	
	Scud (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	24 hours	TL_{50} = 6.5 ppm (1.0 to 8.6 ppm)	60°F, 15.5°C	
		Surface absorption, static bioassay	48 hours	TL_{50} = 5.9 ppm (3.1 to 11 ppm)	--	
		Surface absorption, static bioassay	96 hours	TL_{50} = 5.9 ppm (3.1 to 11 ppm)	--	
Butoxy-ethanol ester of 2,4-D (500 g/liter)	Harpacticoid (Nitocra spinipes; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 3.1 ppm (2.4 to 4.1 ppm)	70 ± 1.8°F, 21 ± 1°C	Linden et al. 1979
Butoxy-ethanol ester of 2,4-D	Pink shrimp (Panaeus duorarum; Crustacea)	Surface absorption	48 hours	No effect observed at 1.0 ppm 2,4-D.	Mortality or paralysis as the endpoint.	Butler 1975
Propylene glycol butyl ether ester of 2,4-D (100%, liquid Esteron®99)	Cladoceran (Simocephalus serrulatus; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 4.9 ppm (4.0 to 6.7 ppm)	First instar, 59°C (15°C)	Sanders 1970
	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 1.2 ppm (0.7 to 2.0 ppm)	Hard water (272 ppm $CaCO_3$). First instar, 70°F (21°C)	
	Seed shrimp (Cypridopsis vidua; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 0.4 ppm (0.3 to 0.7 ppm)	Mature, 70°F (21°C)	
	Scud (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 2.9 ppm (1.7 to 4.7 ppm)	Mature, 59°F (15°C)	
	Glass shrimp (Palaemonetes kadiakensis; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 0.4 ppm (0.09 to 1.4 ppm)	Hard water (272 ppm $CaCO_3$). Mature, 70°F (21°C)	

A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Propylene glycol butyl ether ester of 2,4-D (100%, liquid Esteron®99)	Stonefly (Pteronarcys californica; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 2.6 ppm (1.8 to 3.8 ppm)	Hard water (272 ppm $CaCO_3$). Second year class, 50°F (10°C).	Johnson and Finley 1980
	Stonefly (Pteronarcella badia; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 2.4 ppm (1.9 to 3.2 ppm)	Naiad stage, 50°F (10°C).	
Propylene glycol butyl ether ester of 2,4-D	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 0.1 ppm	70°F, 21°C	Sanders 1970
	Seed shrimp (Cypridopsis vidua; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 0.32 ppm	70°F, 21°C	
	Sowbug (Asellus brevicaudus; Isopod, Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 2.2 ppm	60°F, 15.5°C	
	Glass shrimp (Palaemontes kadiakensis, Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} = 2.7 ppm	70°F, 21°C	
	Crayfish (Oronectes nais; Crayfish)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	60°F, 15.5°C	Sanders 1970
	Scud (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	24 hours	TL_{50} = 4.1 ppm (2.8 to 5.8 ppm)		
			48 hours	TL_{50} = 2.6 ppm (1.7 to 3.9 ppm)		
			96 hours	TL_{50} = 2.5 ppm (1.7 to 3.7 ppm)		
Propylene glycol butyl ether ester of 2,4-D	Pink shrimp (Panaeus duorarum; Crustacea)	Surface exposure	48 hours	No effect observed at 1.0 ppm 2,4-D.	Endpoint was mortality or paralysis.	Butler 1975
Isooctyl (2-ethyl-hexyl) ester of 2,4-D 67%)	Scud (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 2.4 ppm (1.9 to 3.0 ppm)	Mature, 70°F (21°C)	Johnson and Finley 1980

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (analytical grade)	House flies (Musca domestica; Insecta)	Surface absorption (applied topically to ventral surface)	24 hours	Exposure to 15 μg 2,4-D per fly resulted in 1.4-fold increase in mortality with 2,4-D + parathion compared to parathion alone. No change observed in mortality with 2,4-D and DDT compared to DDT alone.	Increase in mortality with parathion + 2,4-D not significant.	Lichtenstein et al. 1973
2,4-D (analytical grade)	Mosquito (Aedes aegypti; Insecta, larvae)	Surface water	24 hours	Exposure to 10 ppm, 2,4-D resulted in 3.2-fold increase in mortality with 2,4-D + parathion and 1.7-fold increase in mortality with 2,4-D + DDT compared to insecticide exposure without 2,4-D.	Increase in mortality with parathion + 2,4-D significant at 99.9% level.	Lichtenstein et al. 1973
2,4-D	Cockroach (Blattella germanica; Insecta)	Oral (ingested with food)	--	When cockroaches were fed 1,000 ppm 2,4-D in food, there were only negligible effects in terms of larval mortality, larval development time, or reproductive potential through the third generation.	--	Riviere 1976
Dimethylamine salt 2,4-D (4 lb per gallon a.e.)	Mayfly (Ephemerella walkeri; Insecta, nymphs)	--	1 hour	At 1.0 ppm, 38% in untreated water; at 100 ppm, 0% in untreated water with no avoidance observed and 70% mortality. No avoidance shown at 10 ppm.	Temperature 32°F (11°C), pH 8.0, hardness 90 ppm as $CaCO_3$, 7.0 ppm dissolved oxygen.	Folmar 1978

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2-Ethylhexl ester of 2,4-D	Brown shrimp (Panaeus aztecus; Crustacea)	Surface absorption	48 hours	Ten percent showed mortality or paralysis at 2.0 ppm.	--	Butler 1975
Butyl ester of 2,4-D (98.4% liquid, Esteron 76-BE)	Stonefly (Peteronarcella badia; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 1.5 ppm (1.2 to 1.9 ppm)	Nymph stage, 50°F, 10°C	Johnson and Finley 1980
2,4-D	Rotifer (Brachionus calyciflorus)	Surface absorption	up to 31 hours	At a 5 ppm concentration, 50% of the test animals died within 24 hours, 100% died within 31 hours.	--	George et al. 1982
2,4-D (acid)	Freshwater oligochaete worm (Lumbriculus variegatus; Annelida)	Surface absorption, static bioassay	48 hours	LC_{50} = 122.2 ppm	Nontoxic concentration of 86.7 ppm.	Bailey and Liu 1980
			96 hours	LC_{50} = 122.2 ppm	Temperature 68 ± 1.8°F (20 ± 1°C)	
2,4-D (acid)	Eastern oyster (Crassostrea virginica; Mollusca)	Surface absorption	96 hours	No effect observed at 2.0 ppm 2,4-D.	Endpoint for effect was a decrease in sheel growth. EC_{50} was the concentration causing a 50% decrease in shell growth.	Butler 1975
Dimethylamine salt of 2,4-D	Eastern oyster (Crassostrea virginica; Mollusca)	Surface absorption	96 hours	No effect observed at 2.0 ppm 2,4-D.		
Butyl ester of 2,4-D	Freshwater Ectoprocta (Plumatella casmiana)	Surface absorption	up to 84 hours	At 4 ppm, individuals were all alive at 12 hours, sluggish at 24 hours, extremely sluggish at 36 hours, and all dead at 48 hours. At 3.0 ppm, individuals were all alive at 12 hours, sluggish at 24 to hours, extremely sluggish at 60 hours, and with 50% mortality at 72 and 84 hours.	At 0.01 ppm, there was no reduction in staoblast germination compared to controls. Reduced germination observed at 1.5 ppm 2,4-D.	Rao and Dad 1979

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Butyl ester of 2,4-D	Freshwater Ectoprocta (Lophopodella carteri)	Surface absorption	up to 48 hours	At 4 ppm, individuals were sluggish at 12 hours, extremely sluggish at 24 hours and all dead at 36 hours. At 3.0 ppm, all individuals remained alive a 84 hours and were not sluggish.	At both 0.01 and 1.5 ppm 2,4-D, reduction in statoblast germination compared to controls was observed.	Rao and Dad 1979
Butyl ester of 2,4-D	Freshwater Ectoprocta (Hyalinella punctata)	Surface absorption	up to 84 hours	At 4 ppm, individuals were all alive at 12 hours, sluggish at 24 hours, extremely sluggish at 36 hours and all dead at 48 hours. At 3.0 ppm, all individuals remained alive at 84 hours and were not sluggish.	—	Rao and Dad 1979
Butoxyethanol ester of 2,4-D	Estern Oyster (Crassostrea virginica; Mollusca)	Surface absorption	96 hours	EC_{50} = 3.75 ppm	Endpoint for effect was a decrease in shell growth. EC_{50} was the concentration causing a 50% decrease in shell growth.	Butler 1965
2-Ethylhexyl ester of 2,4-D	Eastern Oyster (Crassostrea virginica; Mollusca)	Surface absorption	96 hours	Thirty-eight percent growth reduction at 5.0 ppm 2,4-D.	Endpoint for effect was a decrease in shell growth. EC_{50} was the concentration causing a 50% decrease in shell growth.	Butler 1965
Propylene glycol butyl ether ester of 2,4-D	Eastern Oyster (Crassostrea virginica; Mollusca)	Surface absorption	96 hours	Thirty-nine percent growth reduction at 1.0 ppm 2,4-D.	Endpoint for effect was a decrease in shell growth. EC_{50} was the concentration causing a 50% decrease in shell growth.	Butler 1965

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (acid)	Soil microorganisms	2,4-D incorporated into soil cultures	56 days	2,4-D incorporated into soil at a rate equivalent to 5 tons a.i./acre (11,227 kg/ha) in laboratory resulted in 45% inhibition of CO_2 production compared to controls. Bacterial growth was inhibited by both pure 2,4-D and its formulation; streptomyces growth was enhanced with pure 2,4-D and inhibited by its formulation; and fungal growth was enhanced with pure 2,4-D and unaffected by its formulation.	--	Stojanovic et al. 1972
2,4-D	Soil amaeba (Acanthamoeba castellanii)	2,4-D incorporated into culture medium	5 days	Growth and reproduction stimulated at 0.1 and and 1.0 ppm 2,4-D. Less pronounced stimulation at 10 and 100 ppm, with a longer lag phase than controls for 100 ppm 2,4-D.	Acanthamoeba may be able to degrade 2,4-D and use it as a carbon/energy source.	Prescott and Olson 1972
2,4-D (acid)	12 strains of algae	2,4-D incorporated into culture medium	14 days	Low concentrations usually stimulated algal cultures, higher concentrations stimulated or stopped growth. In mixed cultures, most tolerant species decreased toxicity of the herbicide to more sensitive species.	--	Bednarz 1981
2,4-D	Soil fungi	Incorporated into field-collected soil and incubated in laboratory.	40 days	Applied at 1.9, 7.6, and 15.2 ppm in dry soil. Stimulated total count of soil fungi and several fungal species, especially between 5 and 20 days after treatment.	Incubated at 80.6°F (27°C)	Moubasher et al. 1981

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D	Soil fungi	Incorporated in agar medium.	Not given	Stimulated total count of soil fungi as well as Asperigillus sp., A. niger, A. fumigatus, and Fusarium sp. at 6.3 ppm. Toxic at 6.3 ppm to Humicola grisea and Myrothecium verrucaria. At 25.2 and 50.4 ppm, toxic to total fungal counts and to most species.	—	Moubasher et al. 1981
2,4-D	Sewage microorganisms	Incorporated in inoculum	up to 190 hours	Concentrations of 0.1, 1.0, and 100 ppm 2,4-D did not affect rate of O_2 depletion caused by microorganisms degrading organic matter in sewage. Nitrification by an enrichment culture derived from sewage was not affected with 1.4 or 14 ppm 2,4-D. Inocula with 10 ppm 2,4-D did not metabolize significant amounts of herbicide as the sole source of carbon.	--	Lieberman and Alexander 1981 and Rosenberg et al. 1979
2,4-D	Soil microorganisms	Incorporated in organic soil.	2 to 7 days	Concentrations of 5 and 10 ppm (weight) 2,4-D did not significantly alter nonsymbiotic nitrogen fixation, acetylene reduction, or population growth of fungi or bacteria compared to controls.	--	Tu 1979
2,4-D (acid)	Phytoplankton	Surface absorption	4 hours	No decrease in carbon fixation at 1.0 ppm.	--	Butler 1965
2,4-D (99%)	Euglena gracilis (Algae)	Surface absorption	3 days	220 ppm 2,4-D did not adversely affect Euglena cultures in terms of percent motile cells, or population growth as measured by optical density, compared to controls.	Cultured in soil water (slurry) at 77°F (25°C)	Morre 1974 and Elder et al. 1970

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D	Free living Amoebae (23 strains)	Surface absorption	--	28, 54, and 84 ppm 2,4-D did not adversely affect any strain of Amoebae tested.	--	Pons and Pussard 1980
2,4-D	Blue-gree algae (Cyano bacteria)	Incorporated into culture medium	--	Exponential growth rate reduced to 50% of control at 50 to 700 ppm and growth was completely inhibited at 100 to 1,000 ppm.	--	Hutber et al. 1979
Dimethylamine salt of 2,4-D	Phytoplankton	Surface absorption	4 hours	No decrease in carbon fixation at 1.0 ppm.	--	Butler 1965
Butoxyethanol ester of 2,4-D	Phytoplankton	Surface absorption	4 hours	Sixteen percent decrease in carbon fixation at at 1.0 ppm.	--	
2-Ethylhexyl ester of 2,4-D	Phytoplankton	Surface absorption	4 hours	Forty-nine percent decrease in carbon fixation at 1.0 ppm.	--	
Propylene glycol butyl ether ester of 2,4-D	Phytoplankton	Surface absorption	4 hours	Forty-four percent decrease in carbon fixation at 1.0 ppm.	--	
Butoxyethanol ester of 2,4-D (98.2%)	Planktonic algae	Incorporated into culture medium	2 weeks	At a concentration of 0.01 ppm 2,4-D, all 21 strains of algae tested were capable of removing 2,4-D from the culture. Remaining 2,4-D ranged from 13 to 64% of the original amount.	Disappearance of 2,4-D from actively growing culture may not conclusively demonstrate metabolism of 2,4-D by those strains.	Butler et al. 1975a
Butoxyethanol ester of 2,4-D (98.2%)	Planktonic algae (36 isolates)	Incorporated into culture medium	2 weeks	Cultures grown at 0.001, 0.01, 0.1, 1.0, and 4.0 ppm. Concentrations of 1.0 ppm and less did not change population growth patterns of the isolates compared to controls. At 4.0 ppm, some inhibition of growth response occurred (10% of cultures).	--	Butler et al. 1975b

[a] Data not available or not applicable.

[b] 95% confidence limits in parentheses.

Appendix B

Summary of 2,4-D Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (acid, granular, 100%)	Cutthroat trout	Surface absorption, static bioassay	96 hours	LC_{50} = 64 ppm[a] (57-72 ppm)	0.3 gram fish, 50°F (10°C)	Johnson and Finley 1980
2,4-D (acid, granular, 100%)	Lake trout	Surface absorption, static bioassay	96 hours	LC_{50} = 45 ppm (35-56 ppm)	0.3 gram fish, 50°F (10°C)	Johnson and Finley 1980
2,4-D (Technical)	Common carp	Surface absorption, static bioassay	24 hours	TL_{50} = 5.92 ppm (May) to 45.00 ppm (February)	Mean test temperatures ranged from 62.6°F (17°C) in February to 102.2°F) (39°C) in May. Toxicity increased with increase in temperature.	Vardia and Durve 1981
		Surface absorption, static bioassay	48 hours	TL_{50} = 5.80 ppm (May) to 42.50 ppm (February)		Vardia and Durve 1981
		Surface absorption, static bioassay	96 hours	TL_{50} = 5.10 ppm (May) to 35.00 ppm (February)		Vardia and Durve 1981
2,4-D (acid)	Channel catfish	Surface absorption, static bioassay	48 hours	Less than 10% mortality at 10 ppm.	One year old fingerlings (14 grams) were used. Temperature 68 to 69.8°F (20 to 21°C).	McCorkle et al. 1977
2,4-D (acid)	Spot	Surface absorption, static bioassay	48 hours	No observed effect at 50 ppm.	--[b]	Butler 1965
2,4-D (acid)	Pink salmon (fry)	Surface absorption, static bioassay	96 hours	Mean mortality of 10% at 1 ppm, 13.3% at 5 ppm, 43.3% at 10 ppm, and 100% at 50 ppm.	Temperature 50 ± 18°F (10 ± 1°C).	Meehan et al. 1974
2,4-D (acid)	Chum salmon (fry)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 66.7% at 50 ppm. No effect level = 10 ppm.	Temperature 50 ± 18°F (10 ± 1°C).	Meehan et al. 1974

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (acid)	Coho salmon (fry)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 80% at 50 ppm. No effect level = 10 ppm.	Temperature 50 ± 18°F (10 ± 1°C).	Meehan et al. 1974
2,4-D (acid)	Sockeye salmon (smolts)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 6.7% at 50 ppm. No effect level = 10 ppm.		
2,4-D (acid)	Alaska coho salmon (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at concentrations up to 50 ppm.	Temperature 50 ± 1.8°F 10 ± 1°C	Meehan et al. 1974
	Dolly Varden char (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at concentrations up to 50 ppm.		
	Rainbow trout (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at concentrations up to 50 ppm.		
	Oregon coho salmon (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 73.3% at 50 ppm. No effect level = 10 ppm.	Temperature 50 ± 1.8°F (10 ± 1C)	Meehan et al. 1974
2,4-D (acid, 99%)	Green sunfish	Surface absorption	41 hours	No effect on swimming response in fish exposed to 110 ppm a.e.	Temperature 75.2°F (25°C)	Morre 1974; Sergeant et al. 1971 and Sargent et al. 1970
Dimethyl-amine salt of 2,4-D (liquid)	Bluegill	Surface absorption	12 days	At 25 ppm, study terminated at 8 days with no deaths (fry).	NOEL (no death) for small fish = 40 ppm for 12 days.	Hiltibran 1967
	Green sunfish	Surface absorption	—	At 25 ppm, study terminated at 8 days with no deaths (fry).	--	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dimethyl-amine salt 2,4-D (liquid)	Lake chubsucker	Surface absorption	--	At 25 ppm, study terminated at 8 days with no deaths (fry).	--	Hiltibran 1967
	Smallmouth bass	Surface absorption	--	At 25 ppm, study terminated at 8 days with no deaths (fry).	--	
Dimethyl-amine salt of 2,4-D	Coho salmon (yearling fish)	Surface absorption, static bioassay	144 hours	No mortality was observed in fish exposed to 2,4-D at (nominal) concentrations as high as 200 ppm.	Actual measured concentrations were as 237 ppm. No effects on gill ATPase noted and little mortality resulted when fish were placed in seawater for 240 hours.	Lorz et al. 1979
Dimethyl-amine salt 2,4-D (DMA) (liquid, 49%)	Chinook salmon	Surface absorption, static bioassay	96 hours	LC_{50} 100 ppm	1.0 grams fish, 50°F (10°C)	Johnson and Finley 1980
	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} 100 ppm	1.4 gram fish, 50°F (10°C)	
	Fathead minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 335 ppm (245 to 458 ppm)	0.8 gram fish, 62.6°F (17°C)	
	Channel catfish	Surface absorption, static bioassay	96 hours	LC_{50} = 155 ppm (142 to 169 ppm)	1.9 grams fish, 64.4°F (18°C)	
	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 168 ppm (123 to 230 ppm)	1.1 gram fish, 64.4°F (18°C)	Johnson and Finley 1980
	Smallmouth bass	Surface absorption, static bioassay	96 hours	LC_{50} = 236 ppm (185 to 300 ppm)	0.4 gram fish, 50°F (10°C)	
2,4-D amine (Farmco D50)	Mosquito fish	Surface absorption, static bioassay	24 hours 48 hours 96 hours	TL_{50} = 500 ppm TL_{50} = 445 ppm TL_{50} = 405 ppm	69.8 to 71.6°F (21 to 22°C)	Johnson 1978
Dimethyl-amine salt of 2,4-D	Channel catfish	Surface absorption, static bioassay	48 hours	Less than 10% mortality at 10 ppm.	One year old fingerlings (14 grams) were used. Temperature = 68 to 69.8°F (20 to 21°C).	McCorkle et al. 1977

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D-amine (50% a.i.)	Grass carp	Surface absorption, flow-through bioassay	24 hours 48 hours 96 hours	LC_{50} = 3,080 ppm (2,622 to 3,613 ppm) LC_{50} = 2,540 ppm (2,184 to 2,952 ppm) LC_{50} = 1,313 ppm (1,116 to 1,544 ppm)	Fish weight 15.8 ± 8.1 gram. Tests run at 64.4 to 69.8°F (18-21°C), water hardness of 270 ppm as $CaCO_3$, pH 8.1.	Tooby et al. 1980
2,4-D-amine	Major carp	Surface absorption, static bioassay	96 hours	No mortality observed at 100 ppm, the highest dose.	--	Singh and Yadav 1978
Dimethyl-amine salt of 2,4-D	Long-nosed killifish	Surface absorption, static bioassay	48 hours	No effect observed at 15 ppm.	--	Butler 1965
Dimethyl-amine salt of 2,4-D	Rainbow trout	Surface absorption, fish in Y-maze to choose freshwater or water with 2,4-D	1 hour	At 0.1 ppm, 49% of fish in freshwater. At 1.0 ppm and 10.0 ppm, 70% and 75% respectively, in freshwater.	Significantly more fish chose freshwater at 1.0 and 10.0 ppm than expected, showing avoidance reaction at concentrations as low as 1 ppm.	Folmar 1976, 1978
N-oleyl-1,3-propylenediamine salt of 2,4-D (commercial, 57%)	Channel catfish	Surface absorption, static bioassay	96 hours	LC_{50} = 0.3 ppm (0.2 to 0.5 ppm)	1.9 grams fish, 64.4°F (18°C)	Johnson and Finley 1980
	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 0.8 ppm (0.6 to 0.9 ppm)	1.1 grams fish, 64.4°F (18°C)	
Dodecyl (50.7%)/ tetradodecyl (12.7%) amine salt of 2,4-D (oil soluble liquid, 63.4%)	Chinook salmon	Surface absorption, static bioassay	96 hours	LC_{50} = 4.8 ppm (4.0 to 5.8 ppm)	1.0 gram fish, 50°F (10°C)	Johnson and Finley 1980
	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 2.7 ppm (2.2 to 4.3 ppm)	1.4 grams fish, 50°F (10°C)	
	Fathead minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 2.7 ppm (1.8 to 4.0 ppm)	0.9 gram fish, 68°F (20°C)	
	Channel catfish	Surface absorption, static bioassay	96 hours	LC_{50} = 7.0 ppm (5.6 to 8.7 ppm)	0.8 gram fish, 68°F (20°C)	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dodecyl (50.7%)/ teradodecyl (12.7%) amine salt of 2,4-D (oil soluble liquid, 63.4%)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 7.4 ppm (6.1 to 9.0 ppm)	1.4 gram fish, 68°F (20°C)	Johnson and Finley 1980
	Smallmouth bass	Surface absorption, static bioassay	96 hours	LC_{50} = 3.1 ppm (2.6 to 3.7 ppm)	0.4 gram fish, 68°F (20°C)	
Isopropyl-Diethylamol-amine salt (28%)	Green sunfish	Surface absorption	41 hours	No effect on swimming response in fish exposed to 110 ppm a.e.	Temperatures 75.2°F (25°C)	Morre 1974, Sergeant et al. 1971 and Sargent et al. 1970
2,4-D (potassium salt)	Goldfish	Surface absorption, flow-through bioassay	Continuous from fertilization through 4 days post hatch	LC_{50} for 0-days post hatch >187 ppm in soft water (50 ppm $CaCO_3$) and >201 ppm in hard water (200 ppm $CaCO_3$). LC_{50} for 4-days post hatch = 133.1 ppm (108.6 to 174.8 ppm) in soft water and 119.1 ppm (98.5 to 150.6 ppm) in hard water.	In soft water, LC_1 = 8.21 ppm (2.677 to 15.027 ppm) and in hard water is 8.851 ppm (3.835 to 14.637 ppm). At approximately 0.2 ppm 2,4-D, 0 to 1% of hatch were teratic; at appoximately 200 ppm, 11 to 12% of hatch were teratic. At higher concentrations, 73 to 76% of eggs hatched.	Birge et al. 1979
2,4-D (potassium salt)	Largemouth bass	Surface absorption, flow-through bioassay	Continuous from fertilization through 4 days post hatch	LC_{50} for 0-days post hatch = 165.4 ppm (130.6 to 274.1 ppm) in soft water (50 ppm $CaCO_3$) and 160.7 ppm (122.9 to 230.6 ppm) in hard water (200 ppm $CaCO_3$). LC_{50} for 4-days post hatch = 108.6 (92.5 to 138.4 ppm) in soft water and 81.6 ppm (64.8 to 103.5 ppm) in hard water.	In soft water, LC_1 = 13.102 ppm (4.418 to 21.886 ppm) and in hard water is 3.214 ppm (1.218 to 5.989 ppm). At approximately 1.0 ppm 2,4-D 0 to 1% of hatch were teratic; at approximately 180 ppm, 15% of hatch were teratic. At higher concentrations, 49 to 65% of eggs hatched.	Birge et al. 1979

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (potassium salt)	Rainbow trout	Surface absorption, flow-through bioassay	Continuous from fertilization through 4 days post hatch	LC_{50} for 0-days and 4-days post hatch = 11.0 ppm (7.8 to 15.1 ppm) in soft water (50 ppm $CaCO_3$) and 4.2 ppm (2.8 to 5.9 ppm) in hard water.	In soft water, LC_1 = 0.033 ppm (0.009 to 0.084 ppm) and in hard water is 0.022 ppm (0.006 to 0.055 ppm). At approximately 0.05 ppm 2,4-D, 0 to 1% of hatch were teratic; and at approximately 140 ppm, no eggs hatched.	Birge et al. 1979
2,4-D (sodium salt, 80%)	Mullet	Surface absorption, static bioassay	24 hours 48 hours 72 hours 96 hours	LC_{50} = 68 ppm LC_{50} = 47 ppm LC_{50} = 41 ppm LC_{50} = 32 ppm	Fry approximately 30 days old used.	Tag El-Din et al. 1981
2,4-D (sodium salt, 74.7%)	Bleak (Alburnus alburnus eggs)	Surface absorption static bioassay	12 hours 24 hours 36 hours 48 hours	LC_{50} = 159.4 ppm LC_{50} = 129.0 ppm LC_{50} = 63.9 ppm LC_{50} = 12.9 ppm	2,4-D caused delayed embryo development. Normal development was stunted at relatively low concentrations of 2,4-D. 2,4-D was markedly toxic to freshly hatched larvae, and it delayed filling of the swim bladder with air.	Biro 1979
	Bleak (Alburnus alburnus larvae)	Surface absorption, static bioassay	12 hours 24 hours 36 hours 48 hours	LC_{50} = 112.2 ppm LC_{50} = 70.6 ppm LC_{50} = 62.1 ppm LC_{50} = 51.6 ppm		
2,4-D (Li + salt 95%)	Green sunfish	Surface absorption	41 hours	No effect on swimming response in fish exposed to 110 ppm a.e.	Temperature 75.2°F (25°C)	Morre 1974, Sergeant et al. 1971 and Sargent et al. 1970
2,4-D (sodium salt)	Bluegill	Surface absorption	8 days	NOEL (no death) for small fish = 100 ppm for 8 days.	--	Hiltibran 1967
Isooctyl ester of 2,4-D	Pink salmon (fry)	Surface absorption static bioassay	96 hours	Mean mortality of 40% at 1 ppm, 80% at 5 ppm. All fish died at 10 ppm.	Temperature 50 ± 1.8°F (10°C ± 1°C)	Meehan et al. 1974

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Isooctyl ester of 2,4-D	Chum salmon (fry)	Surface absorption, static bioassay	96 hours	No mortality at 1 ppm. Mean mortality of 66.7% at 5 ppm and 60.0 at 10 ppm. All fish died at 50 ppm. No effect level = 1 ppm.	Temperature 50 ± 1.8°F (10 ± 1°C)	Meehan et al. 1974
Isooctyl ester of 2,4-D	Coho salmon (fry)	Surface absorption, static bioassay	96 hours	No mortality at 1 ppm. Mean mortality of 6.67% at 5 ppm and 13.3% at 10 ppm. All fish died at 50 ppm. No effect level = 1 ppm.	Temperature 50 ± 1.8°F (10 ± 1°C)	Meehan et al. 1974
Isooctyl ester of 2,4-D	Sockeye salmon (smolts)	Surface absorption, static bioassay	96 hours	Mean mortality of 6.7% at 1 ppm, 33.3% at 5 ppm, and 63.3% at 10 ppm. All fish died at 50 ppm.	Temperature 50 ± 1.8°F (10 ± 1°C)	Meehan et al. 1974
Isooctyl ester of 2,4-D	Alaska coho salmon (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at 1 or 5 ppm. Mean mortality of 3.3% at 10 ppm 40% at 50 ppm. No effect level = 5 ppm.		
Isooctyl ester of 2,4-D	Dolly Varden (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 66.7% at 50 ppm. No effect level = 10 ppm.	Temperature 50 ± 1.8°F (10 ± 1°C)	Meehan et al. 1974
Isooctyl ester of 2,4-D	Rainbow trout (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 63.3% at 50 ppm. No effect level = 10 ppm.		

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Isooctyl ester of 2,4-D	Oregon coho salmon (fingerlings)	Surface absorption, static bioassay	96 hours	No mortality at 1, 5, or 10 ppm. Mean mortality of 20% at 50 ppm. No effect level = 10 ppm.	Temperature 50 ± 1.8°F (10 ± 1°C)	Meehan et al. 1974
Issoctyl ester of 2,4-D (99.6% ester, 66.1% a.e.)	Cutthroat trout	Surface absorption	96 hours	LC_{50} >50 ppm	Fingerlings, 0.4 to 0.8 grams.	Woodward 1982
Propylene glycol butyl ether ester of 2,4-D	Cutthroat trout	Surface absorption, static bioassay	96 hours	LC_{50} = 1.0 ppm (0.9 to 1.2 ppm)	1.0 gram fish, 50°F (10°C)	Johnson and Finley 1980
	Rainbow trout			LC_{50} = 1.0 ppm (0.8 to 1.1 ppm)	1.5 gram fish 59°F (15°C)	
	Lake trout			LC_{50} = 1.1 ppm (1.0 to 1.2 ppm)	0.6 gram fish, 52°F (10°C)	
	Bluegill			LC_{50} = 0.6 ppm (0.4 to 0.7 ppm)	1.0 gram fish, 64.4°F (18°C)	
Propylene glycol butyl ether ester of 2,4-D	Long-nosed killifish	Surface absorption, static bioassay	48 hours	LC_{50} = 4.5 ppm	--	Butler 1965
Propylene glycol butyl ether ester of 2,4-D (granular)	Bluegill	Surface absorption	12 days	At 5 ppm, fry survived 1 day, at 1 ppm, fry survived 2 days.	NOEL (no death) for small fish = 2.0 ppm for 12 days.	Hiltibran 1967
	Green sunfish	Surface absorption	--	At 1 ppm, fry survived 5 days.	--	
	Lake chubsucker	Surface absorption	--	At 1 ppm, fry survived 5 days.	--	
	Smallmouth bass	Surface absorption	--	At 1 ppm, fry survived 5 days.	--	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Propylene glycol butyl ether ester of 2,4-D (100% ester, 61.7% a.e.)	Cuthroat trout	Surface absorption	96 hours	LC_{50} = 0.77 ppm (0.62 to 0.96 ppm)	Fingerlings, 0.4 to 0.8 grams	Woodward 1982
Butoxy-ethanol ester of 2,4-D (Technical)	Rainbow trout (yearlings)	Surfce absorption	24 hours	LC_{20} = 9 ppm; LC_{50} = 10 ppm; LC_{90} = 10.5 ppm. Following 24-hour exposure to 0.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 ppm, fish displayed an altered response to current with a decrease in positive response frequency at higher concentration and increase in frequency of non-response with increased concentration.	Fish exhibited lethargy at 8 ppm, and all surviving fish at higher concentratin were lethargic. Smallest fish were least affected, largest fish were the first to die. Ester taken up by fish and secreted back into water in the hydrolyzed 2,4-D form.	Dodson and Mayfield 1979
Butoxy-ethanol ester of 2,4-D (Aqua-Kleen commercial formulation ground to a powder, 29% a.i.)	Rainbow trout (yearlings)	Surface absorption	24 hours	LC_{30} = 8.5 ppm; LC_{50} = 9.0 ppm; and LC_{100} ppm = 9.5 ppm (a.i.). Following 24-hour exposure to 0.0, 2,0, 3.0, 4.0, 5.0, 6.0, and 7.0 ppm, no clear relationship was observed between responses to current and exposure concentration.	Fish exhibited lethargy at 7.5 ppm; all surviving fish at higher concentrations were lethargic. Largest fish were the first to exhibit toxic effects.	Dodson and Mayfield 1979
Butoxy-ethanol ester of 2,4-D (Aqua-Kleen commercial formulation whole, 29% a.i.)	Rainbow trout (yearlings)	Surface absorption	24 hours	Seventy percent mortality was observed at 14.0, 15.0, 16.0 and 17.0 ppm concentrations.	--	Dodson and Mayfield 1979

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Butoxy-ethanol ester of 2,4-D (Technical 62.5%)	Fathead minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 3.3 ppm (2.5 to 4.2 ppm)	0.9 gram fish, 64.4°F (18°C)	Johnson and Finley 1980
	Bluegill			LC_{50} = 1.2 ppm (1.1 to 1.3 ppm)	1.4 gram fish, 64.4°F (18°C)	
Butoxy ethyl ester of 2,4-D (500 g a.i./ℓ)	Bleak (Alburnus alburnus)	Surface absorption, static bioassay	96 hours	LC_{50} = 3.2 to 3.7 ppm	50°F (10°C)	Linden et al. 1979
Butoxy-ethanol ester of 2,4-D	Long-nosed killifish	Surface absorption, static bioassay	48 hours	LC_{50} = 5.0 ppm	—	Butler 1965
Butoxy-ethanol ester of 2,4-D	Sockeye salmon	Surface absorption	up to 96 hours	Fry exposed for 96 hours at a concentration of 0.7 and 1.0 ppm had mortality of 100%. Smolts with 100% mortality after 96 hours at 1.0 ppm.	Structural alterations indicative of pathology were recorded in the gill, liver, and interrenal of fry exposed to 0.7 and 1.0 ppm. In smolts, only the interrenal showed changes, which were hypertrophy of interrenal nuclei after 48 hour exposure to 1 ppm. Juvenile fish showed stress response (elevated blood plasma cortisol levels) after 1 hour exposure to 5.0, 15.8, and 50 ppm compared to controls when tested in August and September. Studies in June showed no significant differences. Minimal concentration to induce stress response is only marginally below lethal levels. Smolts stressed due to exposure quickly recovered when placed in uncontaminated water.	McBride et al. 1981

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Buthoxy-ethanol ester of 2,4-D	Bluegill	Surface absorption, static bioassay	up to 1 hour	No change in oxygen consumption was noted in fish exposed to 3 ppm 2,4-D.	Three test temperatures were used: 68°F, 77°F, and 86°F (20°C, 25°C, and 30°C).	Sigmon 1979b
Butoxy-ethanol ester of 2,4-D (43%)	Green sunfish	Surface absorption	—	Toxic response in terms of impaired swimming response within 1 hour in fish exposed to 110 ppm a.e.	Temperature 75.2°F (25°C)	Morre 1974, Sergeant et al. and 1971 Sargent et al. 1970
Butyl ester of 2,4-D (liquid 98.4%)	Cutthroat trout	Surface absorption, static bioassay	96 hours	LC_{50} = 0.9 ppm (0.7 to 1.0 ppm)	0.8 gram fish, 50°F (10°C)	Johnson and Finley 1980
	Lake trout	Surface absorption, static bioassay	96 hours	LC_{50} = 0.9 ppm (0.8 to 1.0 ppm)	0.6 gram fish 50°F (10°C)	
2-Ethyl hexyl ester of 2,4-D	Mullet	Surface absorption, static bioassay	48 hours	No effect observed at 10 ppm.	—	Butler 1965
Butyl ester of 2,4-D	Pink salmon (fry)	Surface absorption, static bioassay	96 hours	All fish died at 1 ppm.	--	Meehan et al. 1974
	Chum salmon (fry)				--	
	Coho salmon (fry)					
Butyl ester of 2,4-D	Sockeye salmon (smolts)	Surface absorption, static bioassay	96 hours	Mean mortality of 70% at 1 ppm, all all fish died at 5 ppm.	--	Meehan et al. 1974
Butyl ester of 2,4-D	Alaska coho salmon (fingerlings)	Surface absorption, static bioassay	96 hours	Mean mortality of 6.7% at 1 ppm, 93.3% at 5 ppm. All fish died at 10 ppm. Mean mortality of 16.7% at 1 ppm. All fish died at 5 ppm.	—	
	Dolly Varden char (fingerlings)	Surface absorption, static bioassay	96 hours			

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Butyl ester of 2,4-D	Rainbow trout (fingerlings)	Surface absorption, static bioassay	96 hours	Mean mortality of 53.3% at 1 ppm. All fish died at 5 ppm.	--	Meehan et al. 1974
Butyl ester of 2,4-D	Oregon coho salmon (fingerlings)	Surface absorption, static bioassay	96 hours	Mean mortality of 26.7% at 1 ppm. All fish died at 5 ppm.	--	
Butyl ester of 2,4-D	Rainbow trout	Surface absorption	up to 4 hours	At 10 ppm (2,4-D) no mortality was observed with 2,4-D alone. Addition of 1 ppm carbaryl resulted in 20 and 80% mortality in 2 and 4 hours, respectively. At 15 ppm 2,4-D, no mortality was observed at 2 hours, 37.5% was observed at 4 hours. With 1 ppm carbaryl, there were 60 and 100% mortality at 2 and 4 hours, respectively. 48 hours exposure to carbaryl alone caused no mortality.	The potentiation of lethal response with carbaryl was blocked by 10 ppm atropine.	Statham and Lech 1975
Butyl ester of 2,4-D (98.4% ester, 78.5% a.e.)	Cutthroat trout	Surface absorption	96 hours	LC_{50} = 0.78 ppm (0.66 to 0.92 ppm)	Fingerlings, 0.4 to 0.8 grams.	Woodward 1982
Isopropyl-butyl esters of 2,4-D (mixed, liquid)	Bluegill	Surface absorption	12 days	At 4 ppm, fry survived 2 days, at 1 ppm, fry survived 5 days.	NOEL (no death) for small fish = 3 ppm for 12 days.	Hiltibran 1967

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Isopropyl-butyl esters of 2,4-D (mixed, liquid)	Green sunfish	Surface absorption	--	At 4 ppm, study terminated at 8 days with no deaths. At 1 ppm fry survived 1 day	--	Hiltibran 1967
	Lake chubsucker	Surface absorption	--	At 4 and 1 ppm, study terminated at 8 days with no deaths (fry).	--	
	Smallmouth bass	Surface absorption	--	At 1 ppm, fry survived 1 day.	--	
2-Ethyl hexyl ester of 2,4-D (granular)	Bluegill	Surface absorption	12 days	At 10 and 5 ppm, study terminated at 8 days with no deaths (fry).	NOEL (no death) for small fish = 50 ppm for 12 days.	Hiltibran 1967
	Green sunfish	Surface absorption	--	At 10 ppm, fry survived 4 days.	--	
	Lake chubsucker	Surface absorption	--	At 5 ppm, study terminated at 8 days with no deaths.	--	
	Smallmouth bass	Surface absorption	--	At 10 ppm, fry survived 5 days.	--	
2,4-D Amine	Anuran Amphibia: Adelotus brevis	Surface absorption	24 hours 48 hours 96 hours	TL_{50} = 255 ppm TL_{50} = 228 ppm TL_{50} = 200 ppm	One to 2 week old tadpoles, 69.8 to 71.6°F (21 to 22°C).	Johnson 1976
	Lymnodynastes peroni	Surface absorption	24 hours 48 hours 96 hours	TL_{50} = 321 ppm TL_{50} = 300 ppm TL_{50} = 287 ppm	One to 2 week old tadpoles, 69.8 to 71.6°F (21 to 22°C).	
	Bufo marinus	Surface absorption	24 hours 48 hours 96 hours	TL_{50} = 346 ppm TL_{50} = 333 ppm TL_{50} = 288 ppm	Two weeks old tadpoles, 69.8 to 71.6°F (21 to 22°C).	Johnson 1976
	Anuran Amphibia: Adelotus brevis	Surface absorption	96 hours	No deaths observed at concentrations of 230, 270, and 340 ppm.	Four week old tadpoles. Data shows increased resistance with age in this species	

[a] 95% confidence limits in parentheses.

[b] Data not available or not applicable.

Appendix C

Summary of 2,4-D Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (acid, Technical)	Mallards (males and females)	Acute oral (by capsule)	--[a]	LD_{50} >>1,000 mg/kg	Animals were 3 to 5 months old.	Tucker and Crabtree 1970
	Pheasants (males)	Acute oral (by capsule)	--	LD_{50} = 472 mg/kg (340 to 654 mg/kg)[b]	Animals were 3 to 4 months old.	
	Coturnix quail (males)	Acute oral (by capsule)	--	LD_{50} = 668 mg/kg (530 to 842 mg/kg)	Animals were 2 months old.	
	Pigeons (males and females)	Acute oral (by capsule)	--	LD_{50} = 668 mg/kg (530 to 842 mg/kg)	Animals ages not given.	
2,4-D (amine, 4 lb a.e. per gallon)	Mallards (males)	Acute oral (by stomach tube)	--	LD_{50} approximately 2,000 mg/kg	Animals were 7 months old.	
2,4-D (acid)	Chickens (male and female)	Administered by intubation, in olive oil.	2 weeks	LD_{50} = 541 mg/kg (358 to 817 mg/kg)	Three-week old chicks.	Rowe and Hymas 1954
2,4-D (isopropyl ester)	Chicks (male and female)	Administered by intubation, in olive oil.	2 weeks	LD_{50} = 1,420 mg/kg (1,127 to 1789)	Three-week old chicks.	
2,4-D (mixed butyl esters)	Chicks (male and female)	Administered by intubation, in corn oil.	2 weeks	LD_{50} = 2,000 mg/kg (1,350 to 2,960)	Three-week old chicks.	
2,4-D (alkanolamine salt)	Chicks	Administered by intubation, in water.	2 weeks	LD_{50} = 380 to 765 mg/kg	Three-week old chicks.	Bjorn and Northen 1948 in Rowe and Hymas 1954
2,4-D (amine)	Chickens (adult hens, 2.2 to 2.9 g New Hampshire)	Given single oral dose of 100, 200 and 300 mg/kg to groups of 3, 6, and 2, respectively.	--	Gastritis. No other adverse clinical or pathological findings.	--	Bjorklund and Erne 1966
2,4-D (sodium salt, Technical)	Mallards (males and females)	Acute oral (by capsule)	--	LD_{50} >>2,025 mg/kg	Animals were 3 to 5 months old.	Tucker and Crabtree 1970

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D (alkanolamine salts of the ethanol and isopropanol series; 2,4-Dow 65% emulsifiable concentrate)	Chicken	Subchronic oral (by pipette)	10 doses	Chickens dosed at a rate of 100 mg/kg per dose did not differ from controls in terms of weight gain. Chickens dosed at 250 and 500 mg/kg per dose had a significantly reduced weight gain (31%) compared to controls (38%).	Dosed at 100, 250, or 500 mg/kg/day for 10 days.	Palmer and Radeleff 1969
2,4-D (acetamide)	Japanese quail	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 14 days old.	Hill et al. 1975
Dimethylamine salt of 2,4-D	Bobwhite	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LD_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 14 days old.	
	Japanses quail	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 10 days old.	
	Ring-necked pheasant	Subchronic, oral (in feed)	5 days in diet followed by 3 days observations	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 14 days old.	Hill et al. 1975
	Mallard	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 10 days old.	
Propylene glycol butyl ether ester of 2,4-D;	Chicken	Subchronic, oral (by capsule)	10 doses	Chickens dosed a rate of 50, 100, and 250 mg/kg per dose showed weight changes of of +49, +19, and -13%, respectively, compared to a 41% weight gain in controls.	--	Palmer and Radeleff 1969

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Butoxyethanol ester of 2,4-D	Bobwhite	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 14 days old.	Hill et al. 1975
	Japanese quail	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Birds 14 days old.	
	Ring-necked pheasant	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 2,500 ppm, 17% mortality observed at 5,000 ppm. Birds 10 days old.	
Butoxyethanol ester of 2,4-D	Mallard	Subchronic, oral (in feed)	5 days in diet followed by 3 days observation	LC_{50} >5,000 ppm	No mortality observed at 5,000 ppm. Bird 10 days old.	Hill et al. 1975
Butoxyethyl 2,4-D	Chicken (females)	Subchronic, oral (in feed)	Fed in diet from age 28 to 48 weeks	Laying hens fed 2,4-D at a rate equivalent to 50 and 150 mg/kg body weight showed no adverse effects of the treatments in terms of rate of egg production, egg or yolk weight egg shell thickness, hatchability, or offspring growth weight.	--	Whitehead and Pettigrew 1972
2,4-D	Chickens (New Hampshire Red)	Maintained 3 chicks per dose with diets containing 0, 500, 1,000, and 3,000 ppm doses.	7 days	Only adverse effect was retarded growth rate at 3,000 ppm.	Chicken weight 210 to 340 gms, 12 chickens.	Rowe and Hymas 1954
2,4-D (amine)	Chicken (New Hampshire, 2.9 kg, adult hens)	Repeated oral dose of 300 mg/kg/day.	up to 24 days	Renal and visceral gout observed in one animal that died on day 5. Slight kidney enlargement in animals sacrificed after 12 and 24 days with no other gross pathological changes.	Chicken weight 2.2 to 2.9 kg.	Bjorklund and Erne 1966

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D	Chickens	Administered 1,000 ppm in drinking water from one day old to maturity. Twenty-nine treated and 25 controls.	--	No adverse effects on weight gain, age at sexual maturity onset of egg production and number of death s due to disease. Caused reductions in number of eggs produced and weight of eggs. One to 2 ppm 2,4-D in egg yolks and trace in egg whites. Increased kidney size and weight. Microscopic pathology of kidney revealed epithelial hypertrophy and hyperplasia of tubular sections with moderate dilatation.	—	Bjorklund and 1966
2,4-D (alkanolamine salt)	Chickens	Twelve doses of 28 to 280 mg/kg/dose.	28 days	No adverse effects at 28 mg/kg dose. Growth depression at 280 mg/kg dose.	--	Bjorn and Northen 1948 in Rowe and Hymas 1954
2,4-D (amine)	Chickens (leghorn)	Fed 500 ppm in standard feed.	up to 18 months	One of 6 died at 5 months from renal gout. Autopsy of other animals revealed acute, waxy muscular degeneration, stomach ulcer, lymphocytic peribronchitis, and anemia.	Sacrificed at 1, 2, 9 and 18 months, 1 to 2 animals per group.	Bjorklund and Erne 1966
2,4-D (amine, Amine 80, 600 g a.i. per liter)	Chicken (eggs)	Aqueous solution sprayed on egg	One exposure 29 hours prior to incubation.	Application of 2,4-D at rates of 1, 10, and 20 times recommended rates had no adverse effect on any parameter used to evaluate either incubation or subsequent live performance.	Incubation performance and evalu- in terms of deaths prior to 18 days incubation, deaths after 18 days incubation but without broken (pipped) shell and deaths after pipping but with failed hatch. Live performance evaluated in terms of weight gain and mortality.	Somers et al. 1973

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D	Chicken (eggs)	Injected into the egg with acetone solvent.	—	2,4-D injected into egg to give concentrations of 10, 50, 100, 200, and 300 ppm in egg with 83, 100, 71, 62, and 0% of control hatch, respectively.	No teratogenic effects in terms of feather blanching were observed.	Dunachie and Fletcher 1970
Isooctyl ester of 2,4-D (4 lb per gallon)	Pheasant (eggs)	Aqueous solution sprayed on eggs.	One exposure of 13 days incubation.	Application of 2,4-D at a rate equivalent to 0.25 lb/acre did not adversely affect egg hatchability, did not cause deformities in chicks, and did not cause death of young hatch from treated eggs.	—	Kopischke 1972
Propylene glycol butyl ether ester of 2,4-D, (Esteron® 99)	Chicken (eggs)	Aqueous solution sprayed on eggs.	One exposure at 0, 4, and 18 days of incubation.	Application at a rate equivalent to 10 lb/acre (11.2 kg/ha) had no adverse effect on any parameter used to evaluate either incubation or subsequent live performance.	Incubation performance evaluated in terms of deaths prior to 6 days incubation, deaths after 6 days incubation but without broken (pipped) shell, deaths after pipping but with failed hatch. Live performance evaluated in terms of weight gain and mortality.	Sommers et al. 1978a
Propylene glycol butyl ether ester of 2,4-D, (Esteron® 99)	Chicken (males and females)	Aqueous solution sprayed on eggs.	One exposure at 0, 4, and 18 days of incubation for 2 successive generations.	Application at a rate equivalent to 10 lb/acre (11.2 kg/ha) had no adverse effect on sperm count or testes weight of males hatched from sprayed eggs compared to controls. Egg weights, and shell strength and porosity of eggs by females hatched from treated eggs did not differ from controls.	Fertilized eggs from females and males hatched from treated eggs, subsequently treated with 2,4-D, did not differ from controls in terms of incubation performance or subsequent live performance.	Somers et al. 1978b

[a] Data not available or not applicable.

[b] 95% confidence limits in parentheses.

Appendix D

Summary of 2,4-D Toxicity in Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity[a]		
2,4-D (acid)	Mice (male)	--[b]	LD_{50} = 375 mg/kg	--	Hill and Carlisle 1947 in International Agency for Research on Cancer 1977
2,4-D (acid)	Mice (male, young adult)	Administered by intubation in olive oil. Observed 2 weeks.	LD_{50} = 368 mg/kg (312 to 434)	--	Rowe and Hymas 1954
2,4-D (acid)	Rats (male, young adult)	Administered by intubation in olive oil. Observed 2 weeks.	LD_{50} = 375 mg/kg (302 to 465)	--	Rowe and Hymas 1954
2,4-D (acid)	Rats	--	LD_{50} > 500 mg/kg	--	McLaughlin 1951
2,4-D (acid)	Rats	--	LD_{50} = 666 mg/kg	--	Hill and Carlisle 1947 in International Agency for Research on Cancer 1977
2,4-D (acid)	Guinea pigs	--	LD_{50} < 320 mg/kg	--	McLaughlin 1951
2,4-D (acid)	Guinea pigs (male and female young adult)	Administered by intubation in olive oil. Observed 2 weeks.	LD_{50} = 469 mg/kg (397 to 553)	--	Rowe and Hymas 1954
2,4-D (acid)	Guinea pigs	--	LD_{50} = 1,000 mg/kg	--	Hill and Carlisle 1947 in International Agency for Research on Cancer 1977
2,4-D (acid)	Rabbits	--	LD_{50} = 800 mg/kg	--	Hill and Carlisle 1947 in International Agency for Research on Cancer 1977 and Spector 1956

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
2,4-D (acid)	Dog (4, young adult)	Administered in a capsule. Observed 2 weeks.	LD_{50} = 100 mg/kg (25 to 250) caused myotonia, anorexia, and weight loss.	--	Drill and Hiratzka 1953 in Rowe and Hymas 1954
2,4-D (acid)	Mule deer	--	LD_{50} = 400 to 800 mg/kg	--	Tucker and Crabtree 1970 in Mullison 1981
2,4-D	Pigs (young)	Fed 500 mg/kg in diet for 2 months.	Developed locomotor disturbances increasing in severity after 1 month; decreased growth rate.	--	International Agency for Research on Cancer 1977 in Lommen 1980
2,4-D (esters of mono-, di- and tripropylene glycol butyl ethers)	Rats (female, young adult)	Administered by intubation in corn oil. Observed 2 weeks.	LD_{50} = 570 mg/kg (510 to 640)	--	Rowe and Hymas 1954
2,4-D (salt and ester formulation)	Sheep	Fed 100 mg/kg/body weight for 481 days.	No adverse effects	--	Rowe and Hymas 1954 in International Agency for Research on Cancer 1977
2,4-D (isopropyl ester)	Mice (male, young adult)	Administered by intubation in olive oil. Observed 2 weeks.	LD_{50} = 541 mg/kg (398 to 736)		
2,4-D (isopropyl ester)	Rats (male and female, young adult)	Administered by intubation in olive oil. Observed 2 weeks.	LD_{50} = 700 mg/kg (569 to 861)	--	Rowe and Hymas 1954
	Guinea Pigs (male, young adult)	Administered by intubation in olive oil. Observed 2 weeks.	LD_{50} = 550 mg/kg (451 to 671)		
2,4-D (mixed butyl esters)	Mice (female, young adult)	Administered by intubation in corn oil. Observed 2 weeks.	LD_{50} = 713 mg/kg (range = 500 to 1,000)	--	Rowe and Hymas 1954
2,4-D (mixed butyl esters)	Rats (female, young adult)	Administered by intubation in corn oil. Observed 2 weeks.	LD_{50} = 620 mg/kg (1,127 to 1,789)	--	

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
2,4-D (mixed butyl esters)	Guinea pigs (female, young adult)	Administered by intubation in corn oil. Observed 2 weeks.	LD_{50} = 848 mg/kg (604 to 1,190)	—	Rowe and Hymas 1954
2,4-D (mixed butyl esters)	Rabbits (female, young adult)	Administered by intubation in corn oil. Observed 2 weeks.	LD_{50} = 424 mg/kg (252 to 712)	—	Rowe and Hymas 1954
2,4-D	Human	Elderly senile male ingested 1 pint of 2,4-D in a kerosene-like solvent.	Death 6 days after ingestion due to ventricular fibrillation induced by cardiac muscle irritability. Tissue concentrations of 2,4-D: blood = 57.60 ppm, brain = 93.40 ppm, kidney = 193.35 ppm, liver = 407.87 ppm and muscle = 117.50 ppm. At autopsy observed perivascular plaques like muscular sclerosis and acute demyelination throughout brain.	—	Minnesota Department of Health 1978
2,4-D (dimethylamine salt)	Human	Swallowed 6 gms or 80 mg/kg	Death preceded by convulsions and vomiting. Congestion in all organs and degenerative changes in nerve cells.	—	Mullison 1981
2,4-D (weed killer containing 36.5% isooctyl ester of 2,4-D and 49% s-ethyl-dipropylthiolcarbamate in water solution)	Human	Swallowed dosage equivalent to 110 mg/kg 2,4-D, 230 mg/kg s-ethyldipropylthiolcarbamate and 2.3 mg/kg epichlorohydrin.	Uncoordiated twitching and paralysis of rib muscles. General muscle damage evidenced by increases in several muscle enzymes and presence of hemoglobin and myoglobin in urine. Complete recovery in several months. Elevated blood SGOT, SGPT, LDH, CPK, and aldolase. Effects due to 2,4-D not epichlorohydrin.	—	Mullison 1981 and Minnesota Department of Health 1978

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
2,4-D (butyl ester)	Mice	--	LD_{50} = 380 mg/kg	--	Konstantinova 1970 in International Agency for Research on Cancer 1977
2,4-D (butyl ester)	Mice	--	LD_{50} = 731 mg/kg	--	Rowe and Hymas 1954
2,4-D (butyl ester)	Rats	--	LD_{50} = 620 mg/kg	--	
2,4-D (butyl ester)	Rats	--	LD_{50} = 1,500 mg/kg	--	Schillinger 1960 in International Agency for Research on Cancer 1977
2,4-D (butyl ester)	Rats	--	LD_{50} = 920 mg/kg	--	Konstantinova 1970 in International Agency for Research on Cancer 1977
2,4-D (butyl ester)	Cats	--	LD_{50} = 820 mg/kg		
2,4-D (butyl ester)	Cattle	--	LD_{50} 100 mg/kg	--	Bjorkland and Erne 1966
2,4-D (butyl ester in water)	Rats	--	LD_{50} = 920 to 1,500 mg/kg	--	Lommen 1980
2,4-D (butyl ester in diesel fuel)	Rats	--	LD_{50} = 300 to 400 mg/kg	--	
2,4-D (butyl ester in water)	Mice	--	LD_{50} = 380 to 640 mg/kg	--	
2,4-D (sodium salt)	Mice, 2	In water.	LD_{50} = 375 mg/kg	--	Hill and Carlisle 1947 and Rowe and Hymas 1954
2,4-D (sodium salt)	Mice	--	LD_{50} = 360 mg/kg	--	Liktionov et al. 1973

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
2,4-D (sodium salt)	Rats	--	LD_{50} = 730 mg/kg	--	Liktionov et al. 1973
2,4-D (sodium salt)	Rats, 2	Administered by intubation in water. Observed 2 weeks.	LD_{50} = 666 mg/kg	--	Hill and Carlisle 1947 in Rowe and Hymas 1954
2,4-D (sodium salt)	Rats (female, young adult)	Administered by intubation in water. Observed 2 weeks.	LD_{50} = 805 mg/kg (610 to 1,063)	--	Rowe and Hymas 1954 in International Agency for Research on Cancer 1977
2,4-D (sodium salt)	Rats	--	LD_{50} = 2,000 mg/kg	--	Schillinger 1960 in International Agency for Research on Cancer
2,4-D (sodium salt)	Guinea pigs (male, young adult)	Administered by intubation in water. Observed 2 weeks.	LD_{50} = 551 mg/kg (417 to 727)	--	Rowe and Hymas 1954
2,4-D (sodium salt)	Guinea pigs, 2	In water.	LD_{50} = 1,000 mg/kg	--	Hill and Carlisle 1947 in Lommen 1980
2,4-D (sodium salt)	Rabbits, 2	In water.	LD_{50} = 800 mg/kg	--	Rowe and Hymas 1954
2,4-D	Monkey	Given up to 428 mg/kg.	At 214 mg/kg no adverse effect. At 428 mg/kg observed vomiting, incoordination, and loss of of muscle tone.	--	Hill and Carlisle 1947 in Dost 1978

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Concluded)		
2,4-D (unspecified)	Human	Swallowed dose not specified.	Doses of 8 to 800 mg/kg/day caused irritation to mouth, throat, and gastrointestinal tract and caused vomiting, chest pain and abdominal pain, diarrhea, muscle twitching, tenderness and, stiffness.	--	U.S. Department Energy 1983; Mullison 1981; and Lommen 1980
2,4-D (alkanolamine salt)	Cattle	Conservative daily oral doses of 50 mg/kg for 112 days, 100 mg/kg for 86 days or 200 mg/kg for 25 days.	No adverse effect at 50 mg/kg. At all other doses observed weight loss, reduced weight gain or illness.	--	Minnesota Department of health 1978
2,4-D (alkanolamine salt)	Sheep	Consecutive daily oral doses of 100 mg/kg for 8 days, 250 mg/kg for 10 days, or 500 mg/kg for 7 days.	No adverse effects at 100 mg/kg. At 250 and 500 mg/kg observed weight loss, reduced weight gain or illness.	--	Minnesota Department of Health 1978
2,4-D (amine)	Pigs	Two pigs each were given single oral doses of 50, 100, 500, or 1,000 mg/kg.	Diarrhea, stilled gait, depression, vomiting, and muscle weakness. Moribund after 24 hours at 2 high doses. Autopsy at 2 to 3 days of survivors revealed gastrointestinal irritation, pneumonia, and renal and hepatic congestion.	--	Bjorklund and Erne 1966
			Toxicity from Human Worker/Exposure		
2,4-D (amine salt and butyl ester)	Human, 248 males and 44 females	Exposure during manufacture for periods of 6 years for 194 persons and 10 years for 98 persons.	Sixty three percent of workers experienced weakness, fatigue, headache, and dizziness. Twenty percent of workers experienced hypertension and bradycardia. Long exposure caused liver dysfunction.	--	Bashirov 1969 in Minnesota Department of Health 1978

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Toxicity from Human Worker/Exposure (Concluded)		
2,4-D (salts and esters)	Human	Exposure during manufacture for 105 workers. Exposure during aerial spraying for 45 workers. Factory air concentration of 2,4-D = 28 to 44 mg/m^3.	Symptoms that developed and and increased with degree of exposure included headaches, fatigue, loss of appetite, pains in stomach and liver, upper respiratory tract problems, and impaired taste sensitivity. Symptoms disappeared by morning.	--	Festisov 1966 in Minnesota Department of Health 1978
2,4-D (sodium, amine and dimethylamine salts)	Human	Workers exposed while thinning out and treating maize fields with dosages from 1.5 to 3 kg/hectare.	One to several days after exposure, workers experienced headache, dizziness, nausea, weakness, eye irritation, nasopharyngeal irritation, burning sensation of the skin, and substernal pain. Workers recovered within days.	--	Radionov 1967 in Minnesota Department of Health 1978
2,4-D	Human	Exposure of 220 workers in a manufacturing plant for 0.5 to 22 years to a concentration of 30 to 40 mg/day.	No adverse clinical effects in comparison with a control population of 4,600 men. No chromosomal effects in ten Karyotyped workers.	--	Johnson 1971 in Minnesota Department of Health 1978
			Acute Dermal Toxicity		
2,4-D	Rats	--	LD_{50} = 1,500 mg/kg	--	Von Rumker and Horay 1972 in Mullison 1981
2,4-D	Rabbits	--	LD_{50} = 1,400 mg/kg	--	Lehman 1952 in Gehring and Betso 1978
2,4-D	Human	Excessive contact, dose not specified.	Skin irritant. Numbness or tingling of extremities, nausea, vomiting, muscle aches, and loss of function.	--	Mullison 1981 and U.S. Department of Energy 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Concluded)		
2,4-D (dimethyl-amine (DMA) salt, isooctyl and butyl esters)	Rabbits	Applied to intact and abraded skin doses of 15 ml 5 times weekly for 3 weeks at concentrations of 0.626 and 3.13%. DMA salt diluted in water. Esters diluted in oil or water. Applications on a soaked gauze patch kept under plastic for 7 hours.	No adverse effects with respect to body weight, survival, hematologic values, clinical chemistry, and organ/body weight ratios. Microscopic examination showed some subepithelial fibrosis and mononuclear infiltration in skin of some animals.	--	Kay et al. 1965 in Gehring and Betso 1978
			Eye Irritation		
DMA 4® (dimethyl-amine salt of 2,4-D)	(unspecified species)	--	Causes eye injury.	--	Mullison 1981
Formula 40® (alkanolamine salt of 2,4-D)	(unspecified species)	--	Causes eye irritation.	--	
Esteron 99® (propylene glycol butyl ether ester of 2,4-D)	(unspecified species)	--	Eye irritant.	--	
2,4-D (highly concentrated solution or wetted material)	(unspecified species)	--	Painful, irritating to conjunctival membranes and may cause corneal damage.	--	Gehring and Betso 1978
			Inhalation Toxicity		
2,4-D	Human	Time weighted average for 8 hour workday, 40 hour workweek repeated exposure.	Airborne TLV = 10 mg/m^3; no adverse effects.	--	TLVs 1983

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Inhalation Toxicity (Concluded)		
2,4-D	Human	Fifteen minute continuous exposure at maximum of 4 times/day separated by 60 minute interval.	TLV short-term exposure limit = 20 mg/m^3; no adverse effects.	--	TLVs 1983
2,4-D	Human	Prolonged breathing of spray. Duration and dose not specified.	Coughing, burning, dizziness, and temporary loss of voluntary muscular coordination.	--	Mullison 1981
2,4-D (sodium salt)	Guinea pigs	Dust inhalation; no details specified.	No adverse systemic effects.	--	Hill and Carlisle 1947 in Rowe and Hymas 1954
			Subchronic Oral Toxicity		
2,4-D	Rats	Fed 1,000 ppm in diet for 1 month.	No adverse effects.	--	Hill and Carlisle 1947 in Rowe and Hymas 1954
2,4-D	Dogs	Fed by capsule at dosages of 2, 5 and 20 mg/kg/day for 5 days/week for 90 days.	No adverse effects at 2 to 10 mg/kg/day. At 20 mg/kg 3 of 4 dogs died and all 4 dogs had stiff hind legs, difficulty swallowing, bleeding gums, necrosis of buccal mucosa, mild liver and kidney changes, and decrease in lymphocytes.	--	Drill and Hiratzka 1953 in Rowe and Hymas 1954
2,4-D (amine salt and ester)	Cattle	Administered 50 mg/kg orally for 112 daily doses.	No adverse effects.	--	Palmer and Radeleff 1969 in Mullison 1981
2,4-D (amine salt and ester)	Sheep	Administered 100 mg/kg orally for 481 daily doses.	No adverse effects, except weight loss in 1 sheep after after 8 doses but became normal with subsequent doses.	--	
2,4-D	Human	Ingested 8 mg/kg/day daily for 3 weeks.	No adverse effects.	--	Mullison 1981

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Continued)		
2,4-D	Guinea pigs	Ten doses of 100 mg/kg over 12 days.	No adverse effects.	--	Hill and Carlisle 1947 in Rowe and Hymas 1954
2,4-D (sodium salt)	Mice	Fed doses up to 93 mg/kg daily for 3 weeks to 3 months.	No adverse effects.	--	Bovey and Young 1981 in U.S. Department of of Energy 1983
2,4-D (sodium salt)	Rats	Fed doses up to 500 mg/kg daily for 30 days.	No adverse effects at doses up to 400 mg/kg. Death at 500 mg/kg.	--	Hill and Carlisle 1947 in U.S. Department of Energy 1983
2,4-D (amine salt)	Rats	Fed doses up to 1,000 ppm daily for 10 months.	No detrimental effects.	--	U.S. Department of Energy 1983
2,4-D	Rats (female, 5 young adults)	Fed doses of 0, 100, 300, 1,000, 3,000, and 10,000 ppm in diet for up to 113 days.	At 0 to 300 ppm, no adverse effects on growth, mortality, food consumption, appearance, BUN, hematological values, and gross and microscopic pathology. At 1,000 ppm, depressed growth rate, excessive mortality, slightly increased liver weight, and slight cloudy swelling of liver. Animals at 3,000 and 10,000 ppm destroyed after 12 days due to food rejection and rapid weight loss. Increased liver and kidney weights and changes in pathology of these organs.	--	Rowe and Hymas 1954
2,4-D (triethanolamine salt or butyl ester)	Pigs (young)	Treated with 50, 100, or 300 mg/kg body weight up to 103 days.	Anorexia and retarded growth in 1 pig given 51 doses of 50 mg/kg triethanolamine salt over 103 days.	--	Bjorklund and Erne 1966 in Minnesota Department of Health 1978

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Concluded)		
2,4-D (triethanol-amine salt)	Pigs (3 castrated males and 2 females, 8 weeks old)	Fed 500 ppm in diet up to 12 months.	Developed locomotor disturbances of increasing severity after 1 month. Normal organ weights and no gross pathological changes at sacrifice. Clinical effects included decreased hematocrit and hemoglobin, reduced albumin and albumin: globulin ratios, and elevated GOT.	--	Bjorklund and Erne 1966
2,4-D	Rats (female, young adult)	Administered by intubation five times a week for 4 weeks doses of 0, 3, 10, 30, 100, and 300 mg/kg. Suspended in corn oil emulsified with 2 ml of 5 to 10% aqueous gum arabic solution. Controls received corn oil only.	At 3 to 30 mg/kg no adverse effects on mortality, growth, behavior, hematological values, BUN, organ weights or gross and microscopic pathology. At 100 mg/kg dose observed gastro-intestinal irritation, cloudy swelling in liver, and depressed growth rate. At 300 mg/kg caused death with gastrointestinal irri-tation the primary adverse effect.	--	Rowe and Hymas 1954
			Subchronic Intravenous/ Abdominal Toxicity		
2,4-D (sodium salt)	Dogs	Intravenous or abdominal injection at rates of 1.17, 2.6, and 3.2 mg/kg for 32 to 37 days.	No evidence of subchronic toxicity.	--	Seabury 1963 in Mullison 1981
2,4-D	Dogs	Six daily intravenous in-jections of 25 mg/kg body weight.	Caused liver damage.	--	Hill and Carlisle 1947 in Minnesota Department of Health 1978
2,4-D	Human	Twenty-one intravenous in-jections of doses from 800 to 960 mg over 32 days. Twenty-second dose was 2,000 mg and 23rd dose was 3,600 mg or 51.4 mg/kg. Total dosage was 16.3 g.	No adverse effects for doses 1 to 22. Highest dose caused stupor, incoordination, weak reflexes, and urinary incontinence for 24 hours then reverted to normal.	--	Mullison 1981

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Dermal Toxicity		
2,4-D (dimethylamine salt)	Rabbits	Gauze patches saturated with 0.313 or 0.626% 2,4-D acid equivalent dosages applied to shaved abraded skin. Fresh patches of the same dosages applied daily to skin 7 hours/day, 5/days/week for 3 weeks.	No significant adverse effects on body weight, survival, hematology, clinical chemistry, organ-to-body weight ratios, or microscopic pathology of the nervous system. Local skin inflammation in control and treated groups.	—	Kay et al. 1965 in Mullison 1981
2,4-D (isooctyl ester)	Rabbits				
2,4-D (butyl ester)	Rabbits				
2,4-D (butyl ester and diethanolamine salt)	Rabbits (albino, white)	Doses up to 3,980 mg/kg applied to shaved skin.	No toxic symptoms.	—	
2,4-D (butoxy ethanol ester)	Rabbits	Not specified.	LD_{50} = 3,980 mg/kg	—	Mullison 1981
			Chronic Toxicity		
2,4-D (99.6% pure with no detectable dioxin)	Dogs (beagle, male and female)	Fed 0, 10, 50, 100, and 500 ppm in diet for 2 years. Three dogs per per sex dose.	One male death at 10 months. All animals normal clinically and with no gross or microscopic abnormal pathology.	--	Hansen et al. 1971 in U.S. Department of Energy 1983
2,4-D (acid)	Rats	Fed doses up to 1,000 ppm daily for 2 years.	No detrimental effects.	--	U.S. Department of Energy 1983
2,4-D [acid (90%) butyl ester (99%), isopropyl ester (99%), isooctyl ester (97%)]	Mice, (C57BL/6 x C3H/Anf)F_1, (C57BL/6 x AKR)F_1, 18 males + (C3H/Anf)F_1 18 females	Groups of 18 mice per sex per strain given single subcutaneous injection of 215 mg/kg 2,4-D in DMSO, 215 mg/kg butyl ester, 100 mg/kg isopropyl ester, or 21.5 mg/kg isooctyl ester in dimethyl sulfoxide at 28 days old and observed up to 78 weeks old.	Survival was 16/18 to 18/18. No significant change in tumor incidence except 5/17 females in (C57BL/6 x AKR)F strain treated with isooctyl ester had significantly increased ($p = 0.01$) reticulum-cell sarcomas.	--	International Agency for Research on Cancer 1977
			Reproductive and Teratogenicity		
2,4-D (acid 9% pure, isopropyl ester = 99% pure, butyl ester = 99% pure, isooctyl ester = 97% pure)	Mice (BL6, AKR, C3H, B6AK, A/HA	Administered unspecified dose orally or subcutaneously during days 6 through 14 of pregnancy.	Increased incidence of fetal anomalies in BL6, AKR, and C_3H strains but not B6AK or A/Ha strains.	—	International Agency for Research on Cancer 1977

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Reproductive and Teratogenicity (Continued)		
2,4-D (acid, propylene glycol butyl ether ester, isooctyl ester)	Rats (Sprague-Dawley)	Administered corn oil suspensions by stomach tube on days 6 through 15 of pregnancy doses up to 87.5 mg/kg/day of 2,4-D acid, 142 mg/kg/day of propylene glycol butyl ether ester, and 131 mg/kg/day of isooctyl ester.	No teratogenic effects at any dose. NOEL = 25 mg/kg/day of 2,4-D acid or equivalent of the esters. No adverse effects on fertility, gestation, viability, or survival of newborne by 2,4-D acid. Observed embryotoxic and fetoxic effects including subcutaneous edema, delayed ossification, decreased fetal weight, lumbar ribs, and wavy ribs at high doses. No adverse effects on fertility and gestation but highest dose of 87.5 mg/kg/day decreased litter size and survival rate of newborne to the end of weaning by 2,4-D esters.	--	Schwetz et al. 1971
2,4-D	Rats	Administered 50% of LD_{50} (estimated dose of 188 mg/kg) once during a critical day of pregnancy.	No teratologic effects. Decreased body weight and increased intrauterine death rate. Caused bleeding in abdominal cavity of fetuses. Enlarged fetal brain cavity.	--	Aleksashina et al. 1973 in Mullison 1981
2,4-D	Rats	Chronic study with dosage of 0.1 to 0.5 mg/kg/day throughout pregnancy.	Reduced size and weight of fetuses and abdominal cavity hemorrhages caused by 0.5 mg/kg/day. No adverse effects at 0.1 mg/kg/day.	--	
2,4-D (acid, dimethylamine salt, butyl ester, isooctyl ester, butoxyethanol ester)	Rats (Wistar)	Administered single oral daily doses of aqueous gelatin or corn oil suspensions of 0 to 150 mg/kg on days 6 through 15 of pregnancy. Females sacrificed and uteri examined on day 22 of pregnancy. Skeletal and visceral anomalies determined in viable fetuses.	Embryotoxic, fetopathic and potentially teratogenic. Caused fetotoxicity such as decreased litter size, fetal weight, and survival of newborne. Increased number of skeletal anomalies including wavy ribs, extra ribs, delayed ossification, and abnormalities in sternum morpology.	--	Khera and McKinley 1972

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Reproductive and Teratogenicity (Continued)		
2,4-D	Rats (Sprague-Dawley)	Fed 1,000 ppm in drinking water during pregnancy and for an additional 10 months. Offspring continued on treatment for 2 years. Began with 10 pregnant females weighing 350 gm. Offspring divided into groups of 10 males plus 12 females for treatment and 9 males plus 8 females for control.	No adverse clinical signs or adverse morphology in parents or offspring. No malformations. Normal pregnancy, birth, and litter sizes. Offspring on drinking water treatment had lower food and water consumptions and lower growth rate than untreated controls. Increased mortality in treated offspring. Normal clinical values and morphology.	--	Bjorklund and Erne 1966
2,4-D (acid, 96.7% pure)	Rats (males and females, Osborne-Mendel	Three generation 6 litter reproduction study. Fed 100 to 1,500 ppm in diet (equivalent to 5 and 75 mg/kg/day). Twenty females and 10 males per test and control group.	No adverse effects on fertility, mean litter size or viability of pups during first 21 days of age at 100 and 500 ppm. At 1,500 ppm observed reduced survival rate of offspring and reduced weanling weight. No adverse effects on litter size or fertility and no birth defects at 1,500 ppm.	--	Hansen et al. 1971
2,4-D	Rats	Females fed doses up to 2,000 mg/kg to 10 animals/group for 95 days then mated with untreated males and continued on dosed diets through gestation and lactation.	Offspring from 2,000 mg/kg females small at birth, 94% died before weanling. Some deaths in offspring from 1,000 mg/kg female group.	--	Gaines and Kimbrough cited in Minnesota Department of Health 1978
2,4-D (acid, isooctyl ester, butyl ester, ispropyl ester)	Hamsters (Syrian golden)	Administered 20 to 100 mg/kg/day by intubation daily on days 6 through 10 of pregnancy. Animals sacrificed on day 14. Resorption sites, corpora lutea, and fetal anomalies evaluated gross and microsopically. Solvent for test materials was acetone: corn oil: carboxymethyl cellulose (1:5.8:10).	No teratologic effects. Infrequent fetal anomalies such as fused ribs developed in ester, but not acid treated groups. These occurred equally in control and treated groups. Fetal viability per litter decreased in one dose 40 mg/kg tested.	--	Collins and Williams 1971 in Mullison 1981

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Reproductive and Teratogenicity (Concluded)		
2,4-D	Sheep	Administered 2 gms (40 mg/kg) by stomach tube daily for 30, 60, and 90 days following breeding.	No birth defects in lambs. No clinical signs of toxicity in ewes during treatment. No histopathological lesions in internal organs of ewes or lambs.	--	Binns and Johnson 1970
2,4-D	Sow	Fed 500 ppm in diet during entire pregnancy. Surviving piglets fed 500 ppm in diet for 8 months.	Loss of appetite. Ten of 15 piglets died within 24 hours after birth. Surviving piglets on treatment had growth depression, persistent anemia, and moderate degenerative changes in liver and kidney. No clincial effects from repeated dosages of 50 mg/kg/day.	--	Bjorklund and Erne 1966
			Carcinogenicity		
2,4-D (acid)	Dogs (male and female)	Fed 0, 10, 50, 100 and 500 ppm in diet for 2 years starting at 6 to 8 months old (equivalent to doses of 0, 0.25, 1.25, 2.5, and 12.5 mg/kg/day of 2,4-D). Three dogs per sex per dose.	Not carcinogenic. Few adverse effects. One female at 100 ppm one male at 10 ppm died. 28/30 survivors at 2 years clinically normal and with no significant lesions.	--	Hansen et al. 1971
2,4-D (amine salt)	Rats	Lifetime feeding studies a of dose of 0.1 of the LD_{50}, 120 male and 45 female rats.	Not carcinogenic. Two treated rats developed tumors, a mammary fibroadenoma and a hemangioma of the mesenterium, at 23 months. One control rat developed a mammary fibroadenoma after 27 months.	--	Arkhipov and Kozlova 1974 in Mullison 1981 and International Agency for Research on Cancer 1977
2,4-D (amine salt)	Mice	Dermal application of 2 drops of 10% solution per week per animal for unspecified length of time.	Not carcinogenic.	--	

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Concluded)		
2,4-D, [acid = 90% pure), isopropyl ester = 99% pure, n-butyl ester = 99% pure, isooctyl ester = 97% pure]	Mice (C57BL/6 x C3H/AnF)F, (C57BL/6 x AKR)F, 18 males and 18 females per strain per test formulation per dose	One strain initially dosed by stomach tube with 46.4 mg/kg 2,4-D acid daily from 7 to 28 days old, then fed 149 ppm in diet until experiment termination at 78 weeks. Second strain initially dosed with 100 mg/kg 2,4-D acid days then fed 323 ppm until termination according to the above identical treatment schedule. Mice treated with isopropyl, n-butyl and isooctyl esters were initially treated with 46.4 mg/kg/day for days 7 to 28 then given diets containing 111, 149, and 130 ppm, respectively, until termination at 78 weeks.	Not carcinogenic. Not tumorigenic.	--	Innes et al. 1969 in Minnesota Department of Health 1978 and International Agency for Research on Cancer 1977
2,4-D [acid = 96.7% pure, no detectable 2.7-dichloro- or 2,3,7,8-tetrachloro-dibenzo-p-dioxin]	Rats (male and female, Osborne-Mendel)	Fed 0, 5, 25, 125, 625 and 1,250 ppm in diet for 2 years. Diets initiated at 3 weeks. Twenty-five rats per sex per dose.	Not carcinogenic. Total number rats with malignant tumors (male and female) for control and treated groups was 6 (control), 8, 7, 7, 8, and 14. Only the 1,250 ppm males had a significantly ($p \leq 0.05$) increased number of malignant tumors 14/25 compared with control males 1/25. No specific tissue targeted by tumors. No adverse effects on growth rate, survival, organ weights, or hematologic values.	--	Hansen et al. 1971 and International Agency for Research on Cancer 1977

[a] 95 percent confidence limits in parentheses.
[b] Data not available or not applicable.

Appendix E

Summary of Mutagenicity Studies with 2,4-D

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D	Salmonella typhimurium	Eight histidine-requiring mutants were treated with a "small crystal" per plate.	Nonmutagenic; no induction of revertant mutants.	--[a]	Andersen et al. 1972
2,4-D	S. typhimurium	Tested a reverse mutation assay with histidine-requiring strains TA1535, TA1536, TA1537, and TA1538. Doses and conditions of test unspecified.	Nonmutagenic; no increase in revertant mutants.	Summary statement only No data presented.	Shirasu 1975
2,4-D	S. typhimurium	Host-mediated assay with strains TA1530 and TA1531 mice given 6 mg (200 mg/kg) by gavage.	Nonmutagenic	--	Zetterberg et al. 1977
2,4-D, Technical (98.6% pure)	S. typhimurium	Strains TA1535, TA1537, TA1538, TA98 and TA100 tested in a reverse mutation assay. 2,4-D tested at concentrations of 10 to 5,000 μg/plate in the presence or absence of metabolic activation.	Nonmutagenic; no increase in number of histidine revertant mutants.	--	Simmon 1979
2,4-D, sodium salt	S. typhimurium	Strains TA1535 and TA1538 tested for reversion to histidine prototrophy. 2,4-D tested at concentrations of 0.03 to 0.0 mg/ml.	Nonmutagenic treatment with 2,4-D at pH 4.3 did not increase number of revertants.	Toxicity to the bacteria was pH dependent.	
2,4-D, sodium salt	S. typhimurium	Strains TA1530 and TA1531 used in a host-mediated assay for back mutation to histidine independence. Mice treated orally with 6 mg 2,4-D. Bacteria injected intraperitoneally. Duration of treatment was 3 hours.	Nonmutagenic. No significant increase in numbers of revertants to prototrophy.	--	Zetterberg et al. 1977

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D	S. typhimurium	In a host-mediated assay, histidine depended mutants were mixed with serum from rats orally treated with an unspecified dose of 2,4-D. The organisms were subsequently examined for back mutations.	Nonmutagenic	--	Styles 1973
2,4-D	Bacillus subtilis	Tested in the Rec- assay with strains H17 Rec+ and M45 Rec- mutants.	Nonmutagenic; no indication of reparable DNA	--	Shirasu 1975
2,4-D, Technical (98.6% pure)	B. subtilis	DNA-proficient and -deficient strains (H17 and M45, respectively) used in a relative toxicity assay. 2,4-D tested at 0.01 to 5 mg/disc.	Postive results obtained at highest dose indicating the interaction of the chemical with DNA.	--	Simmon 1979
2,4-D, Technical (98.6% pure)	Escherichia coli	DNA-proficient and -deficient strains (W3110 and p3478, respectively) used in a relative toxicity assay. 2,4-D tested at 0.01 to 5 mg/disc.	Positive results obtained at highest dose indicating the interaction of the chemical with DNA.	--	Simmon 1979
2,4-D, Technical (98.6% pure)	E. coli	Strain WP2; tested in a reverse mutation assay. 2,4-D tested at concentrations of 1 to 5,000 µg/plate.	Nonmutagenic; no increase in numbers of tryptophan revertant mutants.	--	Simmon 1979
2,4-D	E. coli	Tested in a liquid holding test for forward mutation to streptomycin resistance. Doses and conditions unspecified.	Nonmutagenic	Summary statement only. No data presented.	Fahrig 1974
2,4-D	E. coli	Tested in a forward mutation (Gal Rs) test. Doses and conditions unspecified.	Nonmutagenic	Summary statement only. No data presented.	Fahrig 1974

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D	*E. coli*	Tested in a reverse mutation assay with tryptophan-requiring mutants (B/r WP2 hcr^+, and WP2 hcr^-). Doses and conditions of test unspecified.	Nonmutagenic; no increase in revertant mutants.	Summary statement only. No data	Shirasu 1975
2,4-D	*E. coli*	Strain WP2 try was used. Concentrations and conditions unspecified.	Nonmutagenic	--	Nagy et al. 1975 in IARC 1977
2,4-D	*Saccharomyces cerevisiae*	Host-mediated assay with strain D4. Mice given 6 mg (200 mg/kg).	Nonmutagenic	--	Zetterberg et al. 1977
2,4-D, Technical (98.6% pure)	*S. cerevisiae*	Strain D3; tested in an assay for mitotic recombinants at concentrations of 0.1 to 0.5 percent.	Nonmutagenic; no increase in mitotic recombinants.	--	Simmon 1979
2,4-D, sodium salt	*S. cerevisiae*	Haploid strain RAD18 tested for back mutation to histidine independence. Tested at 0.10 to 0.30 mg/ml.	Mutagenic; mutation to histidine independence occurred at levels of 0.15 mg/ml and above.	Mutation was pH dependent. Mutation occurred at pH values of 4.5 or below.	Zetterberg 1978
2,4-D, sodium salt	*S. cerevisiae*	Strains D4 and D5 used for detection of gene conversion and mitotic recombination, respectively. 2,4-D tested at concentrations of 0.10 to 0.75 mg/ml.	Mutagenic; a dose-dependent increase in frequency of mitotic gene conversion and mitotic recombination observed at pH 4.5 and pH 4.3.	--	Zetterberg et al. 1977
2,4-D, sodium salt	*S. cerevisiae*	Strain D4 used in a host-mediated assay for mitotic gene conversion. Mice treated orally with 6 mg 2,4-D per mouse. Yeast injected intraperitoneally. Duration of treatment was 3 hours.	Nonmutagenic; no significant increase in the frequency of mitotic gene conversion in the trpS and ade 2 locus of strain D4.	--	Zetterberg et al. 1977
2,4-D	*S. cerevisiae*	Tested in a liquid holding test for mitotic gene conversion. Doses and conditions unspecified.	Nonmutagenic.	Summary statement only. No data presented.	Fahrig 1974

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D, as formulation U46 D-fluid	S. cerevisiae	Strain D used in a test for mitotic gene conversions. Yeast cells were treated with herbicide suspension for 16 hours.	Weakly mutagenic. Induced fivefold increase in mitotic gene conversion at the ade 2 and trp 5 gene loci. Considered weakly convertogenic when compared to other known mutagens.	--	Siebert and Lemperle 1974
2,4-D	Serratia marcescens	Tested in a spot test for back mutation. Doses and conditions unspecified.	Nonmutagenic	Summary statement only. No data presented.	Fahrig 1974
2,4-D	Bacteriophage T_4	Cultures treated with 50 μg/plate.	Nonmutagenic; no induction of rII mutants.	—	Andersen et al. 1972
	Bacteriophage mutants AP72 and N17	Cultures treated with 50 μg per plate.	Nonmutagenic; no reversion to T_4 phenotype.	—	
2,4-D, sodium salt (80% a.i.)	Blue-green algae	Cloral populations of Anabsenopsis raciborskii, A. aphanizomenoides, A. spiroides and microcystis flos-aquae, treated with 100 to 1,000 μg/ml 2,4-D for 8 hours.	Nonmutagenic; no increase morphological mutants.	--	Das and Singh 1978
2,4-D	Drosophila melanogaster	Flies fed 4.5 or 9.0 mM in sucrose.	No increase in number of recessive lethals.	—	Vogel and Chandler 1974
2,4-D	D. melanogaster		Slight increase in frequency of recessive lethals.		Magnusson et al. 1977 in Hayes 1982
2,4-D	D. melanogaster	Larvae of genetically stable and unstable strains of D. melanogaster were treated with 25 ppm 2,4-D. Male progeny examined for somatic mutations (pigmented sectors for eye color).	Mutagenic; significant increase ($p < 0.001$) in frequency of somatic mutations in males of the unstable strain. No effect on the stable strain.	--	Rasmuson and Svahlin 1978

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D	Human lymphocytes	Cell exposed in vitro to concentrations ranging from 0.2 to 60 μg/ml for 20 hours. Cells examined for chromosomal aberrations.	Although statistically insignificant, increases in chromosome aberrations (deletions and gaps) were increased at low doses. Statistically significant increase occurred only at concentrations of 50 and 60 μg/ml.	--	Korte and Jalal 1982
2,4-D	Human lymphocytes	Cultured cells exposed to 2,4-D at concentrations ranging from 0.002 to 50 ppm.	An increase in chromosomal aberrations observed at all but the lowest concentrations. Degree of change was independent of dose.	Activity at these low doses raised speculation as to the identity of the active material.	Pilinskaya 1974 in Hayes 1982
2,4-D	Human lymphocytes	Cells exposed in vitro to concentrations ranging from 2 to 60 μg/ml for 48 to 52 hours. Cells examined for sister chromatid exchanges.	Significant increases in ($p<0.05$) rates of sister chromatid exchanges occurred at levels of 10 to 60 μg/ml.	--	Korte and Jalal 1982
2,4-D	Human lymphocytes	Cells treated in vitro and examined for chromosomal aberrations. Doses and conditions unspecified.	Nonmutagenic; no chromosomal aberrations induced.	Summary statement only. No data presented.	Fahrig 1974
2,4-D	Mouse, Bone marrow erythrocytes	Compound tested in a cytogenicity test for the induction of micronuclei. Mice were injected with 100 mg 2,4-D per kg body weight.	Noncytogenetic; no evidence for increase in micronuclei in erythrocytes of bone marrow.	—	Jensen and Renberg 1976; 1980
2,4-D	Mouse, Bone marrow cells	Animals treated with 10 to 300 mg/kg doses.	Significant increase in aberrant metaphases (two-four-fold) in cells from mice treated with 100 to 300 mg/kg doses but not in those from mice receiving 10 to 50 mg/kg. Single fragments were the primary aberration.	--	Pilinskaya 1974 in Hayes 1982

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D	Rat, Bone marrow cells	Oral administration of unspecified dose.	No chromosomal aberrations.	Summary statement only. No data presented.	Styles 1973
2,4-D	Rat Hepatocytes	Cells exposed to chemical and subsequently examined for unscheduled DNA synthesis (UDS).	No evidence of chemically induced DNA repair synthesis.	Summary statement only. No experimental data presented.	Probst and Hill 1981
2,4-D Technical (98.6% pure)	Human embryonic lung cells	Strain WI-38 used in an assay for unscheduled DNA synthesis (UDS). 2,4-D tested at concentrations of 0.1 to 1,000 μg/ml in the presence or absence of metabolic activation.	No evidence for the induction of UDS, indicating no damage and subsequent repair of DNA.	--	Simmon 1979
2,4-D	Mouse, spleen cells	Tested the effect of 2,4-D on Gamma and UV induced DNA-repair synthesis. Cells irradiated cells treated with 10^{-4}M and 5 x 10^{-4}M, 2,4-D.	DNA-repair synthesis was inhibited.	--	Balo-Banga 1973
2,4-D	Bovine cells	Embryonic bovine kidney cells (EBK) and bovine peripheral blood cells (BPB) were exposed to 2,4-D in concentrations from 1 to 1,000 ppm. The cells were then cultured for 6 to 96 hours and examined for chromosomal aberrations and mitotic irregularities.	No chromosomal damage. An EBK cells mutipolar spindles and polyploid mitotic states were observed, suggesting an effect of 2,4-D on the spindle protein and cell division.	--	Bongso and Basrur 1973
2,4-D	Mouse	Animals treated with a single intraperitoneal injection of 125 mg/kg body weight or orally on 5 successive days for a total dose of 75 mg/kg body weight.	No increase in dominant lethal mutations.	--	Epstein et al. 1972

Formulation	Organism	Nature of Exposure	Effects	Comments	References
2,4-D	Barley seeds	Seeds soaked 2,4-D (100 ppm) for 9 hours. Seed and seedlings examined for endpoints.	Increased mitotic and meiotic chromosomal aberrations and poll sterility male generation and induced chlorogebyll-deficient mutations in the M2 generation.	--	Khalatkar and Bhargau 1982
2,4-D	Tobacco plant tissue culture	Tissue cultures of Nicotiana glauca and tumor prone hybrid N. glauca x N. langsdorffii treated with 0.4 ppm 2,4-D.	Clastogenic; induced chromosome aberrations (chromosome breakage) in N. glauca but not in the hybrid.	--	Ronchi et al. 1976
2,4-D	Weeds and cultivated plants	Dose and treatments varied depending upon the plant under investigation.	Chromosome aberrations were induced in a number of plants. The cytological abnormalities included chromosome bridges, fragments, lagging chromosomes, C-mitoses, and chromation bodies.	--	Mohandas and Grant 1972

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with 2,4-D

Formulation	Organism	Dose	Comments	References
2,4-D (^{14}C-labeled and unlabeled)	Cladoceran (Daphnia magna; Crustacea)	Daphnids exposed to 0.1 and 1.0 ppm initial exposure for up to 2 days.	Rapid uptake with near equilibrium reached within 6 hours. Depuration also rapid. At 0.1 ppm in water, tissue residues of 0.265 ppm with 8 hour half-life for 2,4-D depuration in fresh-water. At 1.0 ppm in water, tissue residues of 3.339 ppm with 5 hour half-life for 2,4-D depuration in freshwater.	Ellgehausen et al. 1980
2,4-D (^{14}C-labeled, 98%)	Algae (Chlorella fusca)	Twenty-hour exposure to initial concentration of 0.05 ppm in a static system.	Bioaccumulation factor (wet weight basis) of 6.	Freitag et al. 1982
2,4-D (^{14}C-labeled)	Spiny lobster (Panulirus argus)	Injection 10 mg/kg into the pericardial sinus.	Half-life of 2,4-D in lobster was 6 to 8 hours. Clearance of 2,4-D in hemolymph via the green gland was 3 to 7 times faster than the rate of metabolism in the hepatopancreas. 2,4-D was preferentially taken up by the green gland.	Tuey and James 1980 and James 1979
2,4-D (granular)	Mussels	Aquatic area treated with 1.07 lb/acre (1.2 kg/ha).	Mussels contained 0.38 to 0.70 ppm 2,4-D. Relatively high levels interpreted as reflecting consumption of algae that had absorbed 2,4-D.	Smith and Isom 1967 in Norris et al. 1983
2,4-D (^{14}C-labeled and unlabeled)	Catfish	Catfish exposed to 0.01 ppm initial concentration for up to 4 days.	Half-life of 2,4-D depuration of 1.1 days in freshwater.	Elleghausen et al. 1980

Appendix F (Continued)

Formulation	Organism	Dose	Comments	References
2,4-D (^{14}C-labeled, 98%)	Fish (Golden orfe)	Three-day exposure to 0.05 ppm.	Bioaccumulation factor <10.	Freitag et al. 1982
2,4-D (^{14}C-labeled, 98%)	Rat (male, Wistar, albinos, 170 ± 20 grams at beginning of test)	Animals fed 1 ppm in diet for 7 days, then sacrificed and autopsied on day 8.	Of the applied dose, 99.5% was excreted in urine, 0.9% eliminated in feces, 1.7% detected in carcass, and nondetectable (<0.01%) in fat, liver, or lung. (Reported data does not add up to 100%).	Freitag et al. 1982
2,4-D (acid, ^{14}C-labeled)	Winter flounder	Intramuscular injection of 2.5 or 25 µmol per kg.	For fish injected (with 2.5 µmol/kg, ratios of 2,4-D in tissue to plasma were; bile 43.54; kidney 4.99; gut 2.28; liver 2.04; heart 1.14; skin 1.09; and for remaining tissues <1.00. At 5 hours after dosing, 47.4 to 49.6% of dose was excreted. At 6 hours 52.8% was excreted. Ten percent of excreted material was the taurine conjugate of 2,4-D, the rest was unchanged 2,4-D.	Pritchard and James 1979
2,4-D	Dogfish shark	Intravenous, dose unspecified.	Nearly 60% of dose excreted in urine within 24 hours after injection. Taurine conjugate accounted for 90 to 95% of excreted herbicide.	James and Bend 1976 and Guarino et al. 1977, both in Pritchard and James 1979
2,4-D	Fish	Background environmental levels.	Three percent of 330 samples from 120 locations in Sweden contained detect able residues of 2,4-D ranging from 0.05 to 1.5 ppm.	Erne 1975 in Norris et al. 1983

Formulation	Organism	Dose	Comments	References
2,4-D (granular)	Fish	Aquatic area treated with 1.07 lb/acre (1.2 kg/ha).	Fish did not contain measurable levels (less than 0.04 ppm).	Smith and Isom 1967 in Norris et al. 1983
2,4-D	Miscellaneous Birds and Mammals	Forest applicaton of 2,4-D.	Thirty-one animals trapped 36 to 266 hours after 2,4-D spray. Fifty-nine percent (sic.) contained detectable 2,4-D residues, with average 1.97 ppm ranging from 0.1 ppm to 25 ppm. Average residues declined with time after application, with averages of 6.93 ppm at 1 day, 0.82 ppm at 2 days, and 0.36 ppm at 14 days.	U.S. Department of Agriculture 1977 in Norris 1981
2,4-D (^{14}C-labeled)	Mice (pregnant females, NMRI strain)	Dose of 0.05 mg 2,4-D per mice via intravenous injection.	Mice sacrificed at 5 minutes, 20 minutes, 1 hour, 4 hours, and 24 hours after dosing. 2,4-D showed slight tendency to accumulate in the visceral yolk sac, was passed to the fetus, and was rapidly eliminated from all tissues, including yolk sac, within 24 hours.	Lindquist and Ullberg 1971
2,4-D	Cows and Sheep	Fed 0, 300, 1,000, and 2,000 ppm in diet for 28 days.	With the exception of kidney, tissue residues of 2,4-D averaged less than 1 ppm (in muscle, fat and liver). Higher residues in kidney tissue are due to excretion at unchanged 2,4-D via kidney.	Clarke et al. 1975 in Lommen 1980
2,4-D (acid)	Lactating milk cow	5.5 grams (approximately 10 mg/kg) daily for 106 days. Route assumed to be oral.	Milk without apparent effect on flavor or odor. A calf fed the milk during the last 38 days of production on 2,4-D treated pasture grew normally and in good health. Although 2,4-D was detected in the blood serum of the cow, it was not detected in that of the calf.	Mitchell et al. 1946 in Mullison 1981

Appendix F (Continued)

Formulation	Organism	Dose	Comments	References
2,4-D (^{14}C-labeled)	Rat	Ingestion of up to 100 mg 2,4-D.	Dose appears to influence excretion with highest tissue concentration 6 to 8 hours after ingestion. Rapid decrease in tissue concentration at low doses, with more than 90% eliminated in 24 hours at 1 to 10 mg dose and 40 to 60% elimination in 24 hours at 60 to 100 mg dose with additional 16 to 36% elimination from 24 to 38 hours after treatment.	Khanna and Fang 1966 in Loos 1975 and in Hayes 1982
2,4-D	Man	Ingestion of a single dose of 5 mg/kg.	No detectable clinical effects. Elimination of 2,4-D from plasma occurred by apparent first-order rate process with a half-life of 11.7 hours. Excretion in urine occurred with a half-life of 17.7 hours during which 82.3% was 2,4-D and 12.8% was a conjugate. All absorption via the gastrointestinal tract.	Sauerhoff et al. 1976
2,4-D (99%)	Man	Ingestion of a single dose of 5 mg/kg by capsule.	No detectable clinical effects. Significant levels of 2,4-D in plasma within 1 hour peaking at 30 to 35 ppm 7 to 12 hours after dosing, declining to about 10 ppm at 48 hours and 4 ppm at 168 hours. Plasma clearance half-life of 33.0 $\pm$ 3.1 hours. Urine contained 2,4-D 2 hours after dosing, with 75% of the total dose excreted within 96 hours. No metabolites were detected in any sample.	Kohli et al. 1974
2,4-D	Man	Not reported, lethal.	Tissue concentrations at autopsy were: blood (heart) 400 ppm; blood (peripheral) 260 ppm; brain 45 ppm; liver 150 ppm; and urine 390 ppm.	Curry 1962 in Hayes 1982

Formulation	Organism	Dose	Comments	References
2,4-D	Man	Not reported, lethal.	Tissue concentrations at autopsy were: blood 669 ppm; brain 12.5 ppm; liver 183 ppm; muscle 70 ppm; kidney 63 ppm; urine 270 ppm; spleen 134 ppm; small intestine fat 129 ppm; and large intestine fat 36 ppm.	Nielson et al. 1965 in Hayes 1982
2,4-D	Man	Not reported, lethal.	Tissue concentrations at autopsy were: blood 23 ppm; brain 100 ppm; liver 116 ppm; stomach 56 ppm; small intestine 20 ppm; lung 88 ppm; and heart 63 ppm.	Geldmacher-von Mallinckrodt and Lautenbach 1966 in Hayes 1982
2,4-D	Man	Not reported, lethal.	Tissue concentrations at autopsy were: blood 58 ppm; brain 93 ppm; liver 408 ppm; muscle 118 ppm; and kidney 193 ppm.	Dudley and Tharpar 1972 in Hayes 1982
2,4-D	Man	Not applicable.	Based on 416 samples at human urine from the general population, no samples were tested positive for 2,4-D and only traces of 2,4-D were found.	
2,4-D (^{14}C-labeled)	Man	2,4-D (acid) dissolved in acetone applied both intravenously and topically onto the skin. Topical dose 4 $\mu g/cm^2$, i.v. dose not given.	One hundred percent of the intravenous dose and 5.8% of the dermal dose was excreted in the urine with 120 hours of administration of the 2,4-D.	Feldmann and Maibach 1974 in International Agency for Research in Cancer 1977
Amine salts of of 2,4-D (dodecyl-tetradecyl amine and dimethylamine salts of 2,4-D)	Fish	Initial application of 4 lb a.e./acre (4.48 kg/ha) to 7,000 acres along canal, followed by spot treatment.	Of 60 samples of fish taken along the canal, 3 had residue levels greater than 0.01 ppm (up to 0.162 ppm), 16 had detectable residue levels less than 0.01 ppm, and the rest had no detectable residues.	Schultz and Whitney 1974
2,4-D "Salt"	Fish (3 species)	Exposed to 2.5 ppm in water for 4, 7, or 14 days.	Less than 0.005 ppm 2,4-D in tissue. Unspecified metabolic products ranged from 0.031 to 0.122 ppm.	Schultz 1973 in Norris et al. 1983

Appendix F (Continued)

Formulation	Organism	Dose	Comments	References
Dimethylamine salt of 2,4-D (^{14}C-labeled)	Bluegills	Exposed for up to 7 days to 2 ppm 2,4-D under static conditions at 70°F (21°C) and pH 7.6.	Concentration of ^{14}C expressed as 2,4-D equivalent reached a maximum (whole body) of 0.93 ppm after 24 hours and did not change significantly through the rest of the 7-day period. Edible flesh reached a maximum 0.114 ppm at 96 hours and head/viscera reached a maximum of 2.2 ppm after 24 hours.	Sikka et al. 1977
Dimethylamine salt of 2,4-D (^{14}C-labeled)	Bluegills	Intraperitoneal injection at 1.0 ppm or 2.5 ppm. Fish placed in freshwater and water then monitored for ^{14}C activity.	2,4-D rapidly excreted, with approximately 90% of initial activity excreted within 6 hours of dosing. All of the ^{14}C activity in the excreted material was due to 2,4-D.	Sikka et al. 1977
Dimethylamine salt of 2,4-D (^{14}C-labeled)	Catfish	Exposed for up to 7 days to 2 ppm 2,4-D under static conditions at 70°F (21°C) and pH 7.6.	Concentration of ^{14}C expressed as equivalent reached a maximum (whole body) of 0.20 to 0.25 ppm after 24 hours and through the rest of the 7 days. A major proportion of the 2,4-D was in head/viscera. Only 10% of total radioactivity was in edible flesh.	Sikka et al. 1977
Amine salts of 2,4-D (dodecyl-tetradecyl amine and dimethyl-amine salts of 2,4-D	Common Florida gallinule	Initial application of 4 lb a.e./acre (4.48 kg/ha) to 7,000 acres along canal, followed by spot treatment.	Breast muscle and liver tissue had residue levels of 0.30 ppm and 0.675 ppm, respectively, 1 day after spraying and no detectable residues 4 days after spraying.	Schultz and Whitney 1974
2,4-D salts	Rats, Pigs, Calves and Chickens	Oral administration of 50 to 100 mg/kg.	Administered dose readily absorbed and eliminated, mainly in the urine. Plasma half-lives ranged from 3 to 12 hours--3 hours in rats, 8 hours calves and chickens, and 12 hours in pigs.	Erne 1966a,b both in International Agency for Research on Cancer 1977, Erne 1966c in Hayes 1982

Formulation	Organism	Dose	Comments	References
Propylene glycol butyl ether ester (Esteron® 99 Concentrate)	Man (aerial application crews)	Three crews of 6 persons per crew each made 2 applications about 1 week apart, one with customary clothing and normal precautions, one with protective gear and special hygienic practices. Each crew treated a 100 acre tract at 2 lb a.e./acre each time.	The total internal dose, determined by urine analysis, ranged from nondetectable to 0.0557 mg/kg body weight for normal procedures, to nondetectable to 0.0237 mg/kg body weight for crews using protective gear and special precautions. Estimated respiratory exposure ranged from nondetectable to 0.30 µg/kg body weight for normal procedures, to nondetectable for crews using protective gear and special precautions. Estimated dermal exposure ranged from nondetectable to 0.09 mg/kg body weight for mormal procedures, to nondetectable to 0.02 mg/kg body weight for crews using protective gear and special precautions.	Lavy et al. 1982
Butoxyethanol ester of 2,4-D	Bluegill	Exposed to 3 ppm in water for 8 days.	Mean body concentration of 2,4-D (whole fish) was <0.05 ppm at 68, 77, and 86°F (20, 25, 30°C).	Sigmon 1979b
2,4-D (acid, butyl and isoocty esters)	Mice (C57BL/6) (female)	Subcutaneous injection of 100 mg/kg of 2,4-D acid, butyl or isooctyl esters.	Rate of disappearance from plasma was butyl ester isooctyl ester >2,4-D acid.	Zielinski and Fishbein 1967 in International Agency for Research in Cancer 1977
2,4-D Esters	Cows	Cows allowed to graze on pasture sprayed with "2,4-D esters" at an unspecified rate.	Milk from cows contained 0.01 to 0.09 ppm 2,4-D during first 2 days after spraying, with lower amounts thereafter. Cows put into pasture 4 days after spray had maximum residues of 0.01 ppm in milk.	Klingman et al. 1966 Minnesota Department of Health 1978

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Herbicide Background Statement: 2,4-DP

2,4-DP Section
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2,4-DP Summary

Major Trade Names: Weedone 2,4-DP®; Weedone 170®; Cornex RH®; and Kildip®

Chemical Names: 2-(2,4-dichlorophenoxy) propionic acid

Major Forestry Application: Applied as a postemergence herbicide, usually mixed with 2,4-D, for conifer release, rights-of-way maintence, timber and range management, weed control, engineering, and recreation management.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Toxicity data for invertebrates are unavailable. Algae are resistant to concentrations as high as 10 ppm. Formulations of 2,4-DP are toxic to slightly toxic in the fish species studied. It is mildly toxic in mammals studied, with LD_{50}'s of 400 to 800 mg/kg in mice and rats, respectively. Rats fed a diet containing 12.4 mg/kg body weight for 90 days showed no effect. 2,4-DP has low dermal toxicity, with an LD_{50} of 1,400 mg/kg in mice. There are no conclusive studies demonstrating either mutagenicity or carcinogenicity of 2,4-DP.

Environmental Fate

Very little information is available on the fate of 2,4-D in the environment, but it is assumed to behave in a similar fashion to other phenoxy herbicides. In plants, it would be absorbed by rats and foliage, from which it would translocate to meristematic tissue. In animals, it would not accummulate but would be excreted in unmetabolized form. In soils, it would be moderately mobile, have a short persistence, and be particularly subject to microbial degradation. In water, 2,4-DP would be lost primarily by microbial degradation and by dilution.

Exposure and Hazard

The reported exposure levels at which toxic effects of 2,4-DP have been observed in experimental animals are considerably higher than levels estimated for USDA Forest Service applications.

1.0 General Information

2,4-DP, also known as Dichlorprop and by the trade names* BH® 2,4-DP, Cornox RK®, Desormone®, Desormone® LV 700, Hedonal®, Kildip®, Polymone®, Seritox® 50, U-46®, Weedone 2,4-DP®, and Weedone 170®, is a systemic herbicide used for site preparation, brush control on rangeland and rights-of-way, and for aquatic weed control (Meister Publishing Company 1981). As one of the phenoxy herbicides, 2,4-DP has been formulated with 2,4-D, 2,4,5-T, 2,4,5-TP (Silvex), and MCPA in addition to others (Norris 1981). Like 2,4-D and the other phenoxy herbicides, 2,4-DP interferes with normal plant growth processes. In general, the toxic effects and environmental behavior and fate of 2,4-DP are very similar to other phenoxy herbicides including 2,4-D (U.S. Department of Energy 1983). It is more selective than 2,4-D, however. Very little information is available concerning specific 2,4-DP toxicity and environmental behavior. Therefore, this Herbicide Background Statement is brief and is supplemented by reference to 2,4-D.

1.1 Normal Use Patterns

2,4-DP is used as a postemergence herbicide to control weeds in small-grain cereals, as well as for weed control along rights-of-way in Europe. (Meister Publishing Company 1981 and Weed Science Society of America 1983). It is registered only for noncropland industrial weed control sites such as roadsides, drainage ditchbanks, railroad and utility rights-of-way, and fence rows, as well as foresty applications, in the United States. Typical application rates for weed control in cereals are 2.4 lb/acre (2.7 kg/ha) when used alone, 1.8 to 2.2 lb/acre (2 to 2.5 ka/ha) when mixed with MCPA, and 1.4 to 2.2 lb/acre (1.6 to 2.5 kg/ha) when mixed with benazolin and dicamba (Weed Science Society of America 1983).

The USDA Forest Service uses 2,4-DP, usually mixed with 2,4-D, for conifer release, rights-of-way maintenance, timber and range management, noxious weed control, engineering, and recreation management (Gross 1983). Application rates ranged from 0.6 to 4.7 lb acid equivalent (a.e.) per acre (0.7 to 5.3 kg/ha) for 11 USDA Forest Service Projects in which 2,4-DP (usually mixed with 2,4-D) was used in 1982. Statistics on use of 2,4-DP by the USDA Forest Service are given in Table 1-1.

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service 2,4-DP Use

Purpose	Active Ingredient Per Acre (lbs) Range	Active Ingredient Per Acre (lbs) Average	Acres Treated	Formulation	Application Method
1981					
Aerial					
Conifer release[a]	1.9	1.9	1,062	Liquid	Helicopter
Ground					
Conifer release[a]	0.8 - 1.2	1.1	55	Liquid	Injection
Right-of-Way[b]	0.8 - 2.0	1.7	333	Liquid	Injection and Broadcast
1982					
Aerial[c]					
2 Projects for Timber Management	4.0	4.0	1,185	Liquid	Aerial
Ground (Projects)[d]					
Timber(5), Range (1) Noxious Weeds (1) Engineering (1) Recreation (1)	0.6 - 4.7	2.8	2,253	Liquid	Ground Foliar and Injection

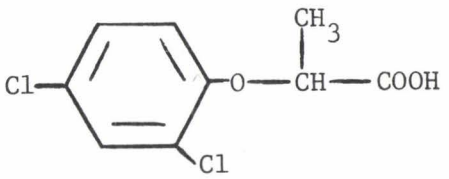
[a] All acres treated with a mixture of 2,4-D and 2,4-DP
[b] 253 Acres treated with a mixture of 2,4-D and 2,4-DP
[c] 1,078 Acres treated with a mixture of 2,4-D and 2,4-DP
[d] 32 Acres treated for engineering, 100 acres for recreation, with a mixture of 2,4-D and 2,4-DP

Source: Gross 1983

Most commercial formulations of 2,4-DP contain the active ingredient as a salt or as an ester (Weed Science Society of America 1983).

1.2 Chemical and Physical Properties

The active ingredient of 2,4-DP is 2-(2,4-dichlorophenoxy) propionic acid, although in commercially available formulations it is generally formulated as a salt or as an ester. The structural formula of 2,4-DP (acid) is (Weed Science Society of America 1983):



2,4-DP has a molecular formula of $C_9H_8Cl_2O$ and a molecular weight of 235.1. In its pure form, 2,4-DP is a white to tan crystalline solid that smells faintly of phenol. It has a melting point of 240.8 to 243.5°F (116 to 117.5°C) and a density of 1.42 (compared to 1.0 for water). It's solubility in water, 0.71 grams per liter (710 ppm), is relatively low. It is more soluble in organic solvents, with values of 595 g/ℓ in acetone; 510 g/ℓ in isopropanol; 85 g/ℓ in benzene; and 2.1 g/ℓ in kerosene at 82.4°F (28°C) (Weed Science Society of America 1983).

Loos (1975) and Norris (1981) reviewed degradation of the phenoxyacetic acids including 2,4-DP. Degradation and loss of 2,4-DP occurs by photodecomposition, volatilization, and metabolism by microorganisms and plants.

2.0 Toxicity of 2,4-DP

The toxicological properties of 2,4-DP, summarized in Table 2-1, indicate that the formulations tested are toxic to slightly toxic to those species tested. Data on carcinogenicity and mutagenicity of 2,4-DP are lacking. It should be noted, however, that as with 2,4-D (see Herbicide Background Statement for 2,4-D), there is no conclusive evidence that 2,4-DP is carcinogenic or mutagenic.

2.1 Invertebrates and Microorganisms

Invertebrates and microorganisms showed a wide range of toxic responses to 2,4-D, generally with a greater sensitivity to ester formulations (Table 2-2 in 2,4-D Herbicide Background Statement). The toxicity of 2,4-DP is expected to be similar to that of 2,4-D, with only slightly toxic effects in most insects; toxic to slightly toxic effects (using salt and acid formulations) to microorganisms, crustacea, and other invertebrates; and toxic to highly toxic effects (using ester formulations) to microorganisms, crustacea, and other invertebrates. Seventeen strains of algae, tested with 2,4-DP, were all resistant to herbicide concentrations of up to 10 ppm with 30-days exposure in medium containing the herbicide (Cullimore 1975).

2.2 Fish

Toxicity data for exposure of fish to 2,4-DP have been reported for bluegill at various growth stages (Hiltibran 1967 and U.S. Department of the Interior 1968 in Pimentel 1971) and for lake chubsucker fry (Hiltibran 1969). Granular 2,4-DP as the isoctyl ester was toxic to adult bluegill (48-hour LC_{50} = 1.1 ppm), slightly toxic to small bluegill and bluegill fry (no deaths at 20 and 10 ppm, respectively), and potentially toxic to lake chubsucker fry (no deaths at 1.5 ppm, the highest dose tested).

Clarke et al. (1970) suggest that pesticides with median lethal concentrations of 10 ppm or more for fish be considered "slightly toxic," between 1 and 10 ppm be considered "toxic," and less than 1 ppm be considered "highly toxic." Assuming that fish responses to 2,4-DP are similar to fish responses to 2,4-D, acid and salt formulations of 2,4-DP should be considered slightly toxic and esters should be considered highly toxic to fish.

2.3 Birds

The toxicity of 2,4-DP to birds has been studied by injection of 2,4-DP into chicken eggs. At 100 ppm, 2,4-DP resulted in reduction of hatching (70 percent of controls), but at 200 ppm, complete inhibition of hatching occurred (Dunachie and Fletcher 1970). No other data are available; however, assuming bird response to 2,4-DP is similar to response to 2,4-D, 2,4-DP has a low toxicity to birds.

Table 2-1

Summary of Toxicological Properties of 2,4-DP[a]

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-DP (acid)	17 strains of algae (Chlorophycae)	Incorporated into culture	Up to 30 days	All strains were resistant to concentrations as high as 10 ppm (highest level tested)	Three strains showed stimulation of growth at 10 ppm.	Cullimore 1975
Isooctyl ester of 2,4-DP (Granular)	Bluegill (small fish)	Immersion	12 days	No deaths at 20 ppm	--[b]	
	Bluegill (fry)	Immersion	10 days	No deaths at 10 ppm (highest level tested)	--	Hiltibran 1967
	Lake Chub-sucker (fry)	Immersion	10 days	No deaths at 1.5 ppm (highest level tested)	--	
2,4-DP (Formulation not specified)	Bluegill	Immersion	48 hours	LC_{50} = 1.1 ppm	--	U.S. Department of the Interior 1968 in Pimentel 1971
2,4-DP	Chickens (eggs)	Injected into egg	--	Injected to give 10, 50, 100, and 200 ppm in egg. Significantly fewer eggs hatched (70% of controls) at 100 ppm, none hatched at 200 ppm.	--	Dunachie and Fletcher 1970

Table 2-1 (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-DP	Mice	Acute oral	--	LD_{50} = 400 mg/kg	--	Neumeyer et al. 1969 in Pimentel 1971
2,4-DP	Rats	Acute oral	--	LD_{50} = 800 mg/kg	--	Weed Science Society of America 1983
2,4-DP	Rats	Chronic oral	90 days	No effect noted when fed 12.4 mg/kg daily	--	Weed Science Society of America 1983
2,4-DP	Mice	Dermal	--	LD_{50} = 1,400 mg/kg	A 2.4% solution did not irritate skin	Weed Science Society of America 1983
2,4-DP	Mice	Eyes	--	1% solution did not irritate eyes	--	Weed Science Society of America 1983

[a] Due to lack of specific studies on 2,4-DP toxicity, no Appendices are included in this background statement. Appendices A, B, C, D, and E of the 2,4-D Herbicide Background Statement contain toxicity data discussed in this section.

[b] Data are not available or not applicable.

2.4 Mammals

Studies of toxic effects of 2,4-DP in mammals indicate an acute oral LD_{50} of 400 to 800 mg/kg body weight (in mice and rats, respectively) (Neumeyer et al. 1969 in Pimentel 1971 and Weed Science Society of America 1983), no effect in rats fed 12.4 mg/kg for 90 days (Weed Science Society of America 1983), a dermal LD_{50} of 1,400 mg/kg in mice (Weed Science Society of America 1983), and no eye irritation with a 1 percent solution (Weed Science Society of America 1983). These toxicity data are in the same range as data reported in the Herbicide Background Statement for 2,4-D. Assuming mammalian response is similar to both 2,4-D and 2,4-DP, 2,4-DP would be considered mildly toxic (causing gastrointestinal disturbances, weight loss, muscle weakness, and lack of coordination); would have the potential for mild eye, skin, and respiratory irritation; would be potentially teratogenic; and would have potential for embryo- and fetotoxicity.

There are no conclusive studies demonstrating carcinogenicity of 2,4-DP. Based on long-term studies in rats, mice, and dogs with several formulations, 2,4-D is a suspect carcinogen. However, there are no conclusive data demonstrating the carcinogenicity of 2,4-D.

2.5 Mutagenicity

There are no conclusive studies demonstrating mutagenicity of 2,4-DP. Mutagenicity of 2,4-D has been reviewed extensively, however (Hayes 1982 and Mullison 1981). In most microbial systems tested, 2,4-D was nonmutagenic. In a yeast study, evidence of mutagenicity appeared only at a low pH (4.5 or below). Increased chromosomal aberrations were observed in several plant studies with 2,4-D. Section 2.5 of the 2,4-D Herbicide Background Statement reviews 2,4-D mutagenicity studies.

3.0 Environmental Fate

2,4-DP and its formulations are postemergence herbicides used to control broadleaf weeds in industrial sites and rights-of-way, and as brush control agents in forestry applications (Meister Publishing Company 1981 and Weed Science Society of America 1983). Although 2,4-D persistence in soil is short (less than 1 month) (Ashton 1982), Burger et al. (1962 in Pimentel 1971) report a moderate persistence of 2,4-DP (103 days). It has a variable toxicity to fish and aquatic invertebrates depending upon formulation, environmental parameters, and species, and generally has a low to moderate toxicity to most other animal species.

Loos (1975) reviewed plant uptake, mode of action, and degradation of phenoxy herbicides. Assuming that 2,4-DP behaves generally like other phenoxy herbicides, it is absorbed by both plant roots and foliage and translocated up and down the plant stem. The major sites for accumulation are in meristematic tissues. Phenoxy herbicides are readily metabolized in plants by a variety of metabolic pathways. In animals, they tend to be readily excreted in urine and do not bioaccumulate.

3.1 Bioaccumulation and Metabolism

Bioaccumulation and metabolism of phenoxy herbicides in animals, plants, and microorganisms are extensively reviewed in Loos (1975). Specific reviews of 2,4-D behavior in plants and/or animals by Ghassemi et al. (1981), Mullison (1981), Hayes (1982) and in these Herbicide Background Statements provide information on the probable behavior of 2,4-DP. The following discussions of metabolism and bioaccumulation in plants and animals assume that 2,4-D and 2,4-DP behave similarly and are based primarily on Loos (1975).

3.1.1 Plants

Plant uptake of phenoxy herbicides occurs through leaves, stems, and roots with the waxy cuticular surface of the plant being the primary limiting factor in herbicide uptake. This cuticular barrier can be overcome by use of lipid soluble formulations such as esters, by use of surfactants, or by application through the bark or cuticle by injection or hack and squirt techniques.

Once absorbed by the plant, translocation of phenoxy herbicide depends upon site of uptake, environmental conditions of moisture and temperature, and specific physiological status of the plant. Translocation of phenoxy herbicides usually takes place readily, with the herbicides being carried along with products of photosynthesis as they are translocated upward from roots in the transpiration stream.

Herbicidal activity of phenoxy herbicides is due to their interference with normal plant growth processes. In addition to imitating normal plant growth hormones (auxins), phenoxy herbicides tend to persist in plant tissue for a longer period and to have a different distribution then natural hormones, thus disrupting normal growth processes.

Plants readily metabolize phenoxy herbicides by changing the molecular structure of the compounds. Resistance of plants generally results from their ability to metabolize the phenoxy herbicide to a nontoxic metabolite.

3.1.2 Animals

Animals do not bioaccumulate phenoxy herbicides to any great extent. Any absorbed material is usually excreted in unmetabolized form. Various studies with 2,4-D, discussed in Section 3.1.2 of the 2,4-D Herbicide Background Statement, indicate that residues of 2,4-D in humans and animals are low following exposure and are virtually nonexistent in terms of background levels. Due to the low proportional use of 2,4-DP compared to 2,4-D, residues of 2,4-DP should be negligible.

3.2 Soils

The fate and transport of 2,4-D and/or phenoxy herbicides, have been reviewed by several authors including Ghassemi et al. (1981) and Norris (1981). Loos (1975) discusses in detail the effect of soil microorganisms on phenoxy herbicides.

The persistence of phenoxy herbicides in soil is generally short, with herbicidal effects lasting only a month. Burger et al. (1962 in Pimentel 1971) report that 2,4-DP (dichlorprop) applied at 25 ppm persisted in soil for more than 103 days. Altom and Stritzke (1973) reported a 10-day average half-life for 2,4-DP in Oklahoma soils in a laboratory study. Smith (1978) also reported a 10-day average half-life for 2,4-DP in Saskatchewan soils.

Assuming that the behavior of 2,4-DP and 2,4-D in soils is similar, 2,4-DP would be relatively mobile compared with most other herbicides. Mobility is influenced by the specific water solubility of the 2,4-DP formulation and the organic content and pH of the soil. The major route of degradation of 2,4-DP would be microbial degradation, especially in warm, moist soils with high organic matter content. Photodegradation would play a minor role in loss of 2,4-DP and volatilization would depend on the volatility of the specific formulation.

3.3 Water

Behavior of phenoxy herbicides in water is reviewed by Norris (1981). Assuming similar behavior of 2,4-D and 2,4-DP in water, the persistence of 2,4-DP would depend upon the presence of microorganisms capable of biodegrading 2,4-DP. Although photodecomposition of 2,4-D has been demonstrated in the laboratory, the actual extent to which photodecomposition of 2,4-DP would occur in the field is unknown. Volatilization should not play a major role in loss of 2,4-DP in water (Ghassemi et al. 1981).

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to 2,4-DP during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved 2,4-DP. Calculations of exposure to 2,4-DP in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to 2,4-DP are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service 2,4-DP Application

Available application data for 2,4-DP use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to 2,4-DP differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of 2,4-DP in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

In general, 2,4-DP is rapidly degraded in the environment and does not bioaccumulate. Consequently, the potential for incidental exposure of humans and wildlife to 2,4-DP is low, existing only for a very short period immediately after its application.

4.2 Exposure

Estimates of potential exposure to 2,4-DP are based on aerial as well as ground application. The maximum aerial application rate for 2,4-DP (see Table 1-1) is 4.0 lb a.e./acre, while the compound is applied from the ground (ground foliar) at rates as high as 4.7 lb a.e./acre. In both aerial application and in ground foliar application, 2,4-DP is applied as a liquid. The calculations in Table 4.2 that are based on aerial application, (e.g., spray/observer dose,

Table 4-1

USDA Forest Service Application Data for 2,4-DP (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
2,4-DP (9)	Ground Foliar and Injection	7.7	70	5.9	13.6	7.1	7.1	Timber (5), Range (1) Noxious Weeds (1) Engineering (1) Recreation (1)
2,4-DP (2)	Aerial	29.5	59	2.5	2.5	8.5	1.5	Timber (2)

[a]Grand Total = 129

Source: Gross 1983

Table 4-2

Summary of Occupational Dose, Environmental Exposure, and Toxicity for 2,4-DP

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg[b]		
Observer	up to 0.16 mg/kg		
Backpack Sprayer	0.188 mg/kg		
Environmental Exposure			
Fish	0.04 ppm (Immersion)[c]		
Rabbits	1.612 mg/kg	Negligible	4.94 mg/kg
Deer	0.404 mg/kg	Negligible	6.77 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.0008 mg/kg (2 days)
(Rabbits)	NA	NA	0.023 mg/kg (2 days)
(Deer)	NA	NA	0.028 mg/kg (2 days)
(Water)	NA	NA	0.0016 mg/kg (1 day)
(Berries)	NA	NA	0.004 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0020 mg/kg (1 day)
Toxicity Summary	LD_{50} = 1,400 mg/kg for mice	None available	Acute LD_{50} = 400 (mice) to 800 (rats) mg/kg
	Most studies, LC_{50} >1.0 ppm for fish		

[a]Assumes aerial application at 4.0 lb active ingredient per acre and ground foliar application at 4.7 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure and are on a daily basis.

[c]Fish exposure results from immersion in water containing 2,4-DP.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

wildlife dermal exposures, and water concentration) assume an application rate of 4.0 lb a.e./acre. Calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose and wildlife oral exposures) assume an application rate of 4.7 lb a.e./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of 2,4-DP and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the exposure estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Lavy et al. (1982) have estimated 2,4-D doses as high as 0.0557 mg/kg body weight for aerial applicators treating forests with 2,4-D at 2 lb a.e./acre. These figures are based on urinalyses of workers using normal precautions and customary clothing. This figure is lower than the value used for the occupational doses for the other herbicides in this study, but it is within a factor of two. Therefore, this analysis will utilize the more conservative dose estimates for mixer/loader, observer, and backpack sprayer discussed in Section 4 of the General Introduction to the Herbicide Background Statements. As indicated in the General Introduction, urinary output was used to calculate total dose (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for 2,4-DP are based on exposures on a per pound per acre application rate multiplied by 4.0 lb/acre for observers and 4.7 lb/acre for backpack sprayers. In the case of the aerial spray observer, exposure estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. At 4.0 lb a.e./acre application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.16 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 4.7 lb/acre application rates, the following estimate of daily doses is derived:

Backpack Sprayer: 0.188 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to independent of application rate and have been estimated to be 0.1 mg/kg.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by 2,4-DP application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less (by orders of magnitude) than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 4.0 lb a.e./acre aerial spray:

Rabbits in Aerial Spray Zone: 1.612 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.404 mg/kg (0.101 mg/kg at 1 lb acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. This exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately

100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of 2,4-DP in water, calculated as:

Water concentration: 0.04 ppm (0.01 ppm at 1 lb/acre application rate)

This concentration of 2,4-DP represents the estimated maximum concentration immediately after spraying. If heavy rainfall occurred immediately after spraying, higher transient concentrations could occur in streams and ponds. However, such high concentrations result from high application rates or result from precipitation soon after application, and would be transient since they are rapidly diluted by resident water.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of 2,4-DP by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 4.7 lb a.e./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 6.768 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbits: 4.935 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of 2,4-DP in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.04 ppm

- bioconcentration: 1.0 (actual bioconcentration for 2,4-D, another phenoxy herbicides, is < 1.0 in most studies)

Fish tissue concentration = 0.04 ppm (0.04 mg/kg)

Deer and rabbit tissue concentrations include 2,4-DP accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied 2,4-DP is absorbed at a rate of 10 percent. It is also assumed that oral exposure of deer and rabbit to 2,4-DP lasts only for 2 days due to the relatively short

environmental persistence of 2,4-DP and that 10 percent of the ingested material is assimilated. Deer tissue concentrations of 2,4-DP are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.404 mg/kg (dermal exposure) x 0.1 = 0.0404 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 6.768 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.3536 mg/kg.

Total Deer Tissue concentration = 0.0404 mg/kg + 1.3536 mg/kg = 1.3940 mg/kg (1.3940 ppm).

Rabbit tissue concentrations of 2,4-DP are derived in the same ways as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 1.612 mg/kg (dermal exposure) x 0.1 = 0.1612 mg/kg

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 4.935 mg/kg/day (oral exposure) x 0.1 x 2 days = 0.9870 mg/kg.

Total Rabbit Tissue Concentration = 0.1612 mg/kg + 0.9870 mg/kg = 1.1482 mg/kg (1.1482 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to 2,4-DP from eating meat are derived:

Fish meat: 0.04 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0008 mg/kg

Deer meat: 1.3940 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0279 mg/kg)

Rabbit meat: 1.1482 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0230 mg/kg

Water and plant consumption by man would result in the following oral exposures to 2,4-DP based on assumptions stated in the introductory chapter of the Herbicide Background Statements at an application rate of 4.0 lb a.i./acre (aerial):

Water: 0.0016 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries: 0.004 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms: 0.0020 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of 2,4-DP to fish and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares exposure estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information. The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for USDA Forest Service applications.

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Herbicide Background Statement: Dalapon

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Dalapon Summary

Trade Name: Dowpon® M

Chemical name: 2,2-dichloropropionic acid; formulated as the sodium and magnesium salts of 2,2-dichloropropionic acid

Major Forestry Applications: Grass control, conifer release, site preparation, timber management, and rights-of-way maintenance.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Dalapon is generally nontoxic to a variety of nontarget organisms and has low toxicity for most animal species. Except for some aquatic crustacea and insects, it is only slightly toxic to most invertebrates and microorganisms. Dalapon generally has low toxicity to various soil microorganisms. Dalapon is only slightly toxic to fish and amphibians. Median lethal concentrations generally are greater than 100 ppm for fish exposed to dalapon for 24 hours or more.

Dietary studies with dalapon indicate a low toxicity to birds, although reproductive effects were reported when dalapon was administstered at high doses to birds. Decreased rates of hatching were reported when chicken eggs were injected at doses giving 300 ppm dalapon in the eggs. No teratogenic effects were observed.

The relatively low order of acute toxicity of dalapon sodium salt and dalapon formulations in mammals is reflected in oral LD_{50}'s from 9300 mg/kg for dalapon sodium administered to rats to 2140 mg/kg for Dowpon®M administered to rabbits.

Tests in rabbits show that dalapon sodium salt is moderately irritating to the eye with transient corneal injury and is moderately irritating to the skin. There is no evidence that dalapon sodium is absorbed through the skin in toxic amounts.

No deaths occurred in rats fed diets containing as much as 1150 mg dalapon sodium salt per kg body weight for 97 days. There were no adverse effects at levels of 115 mg/kg. No adverse effects on reproduction occurred in rats treated with up to 300 mg dalapon/kg/day. No teratogenic effects were noted in rats treated with up to 2000 mg/kg/day.

Dalapon sodium salt was not reported to be carcinogenic on two year studies in dogs and rats. Mutagenicity testing of dalapon has not been reported.

Enviromental Fate

Dalapon is absorbed by plant roots and foliage. It is readily translocated throughout the plant and accumulates in areas of new growth, budding, and fruiting activity. It is not readily metabolized in plants. In animals, it is hydrolyzed to a normal metabolite which is further degraded to acetate and carbon dioxide. Dalapon is rapidly excreted in the urine of animals when ingested, and very few residues are found in tissues.

Dalapon does not persist in the soil. It is very mobile and is readily leached from the soil. It is broken down rapidly and completely by soil microorganisms and biodegradation is the major means by which dalapon is lost from soil. Dalapon is highly soluble in water and is primarily lost in waterbodies by microbial degradation and dilution.

Exposure and Hazard

The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for USDA Forest Service applications. Generally, there should be no significant potential for adverse reproductive or carcinogenic affects to individuals from dalapon use, if proper care is taken during application.

1.0 General Information

1.1 Normal Use Patterns

Dalapon, also known by the trade names* Dowpon® M, is a herbicide that is moderately selective for annual and perennial grasses. It was originally introduced in 1953 by the Dow Chemical Company and has also been marketed under the trade names Dowpon®, Dowpon® C Improved, and Radapon® Liquid. Since its introduction, it has been widely used in a variety of applications for grass control in agriculture, rights-of-way maintenance, industry, and forestry (Ghassemi et al. 1981 and Weed Science Society of America 1983). Dalapon formulations are particularly effective against quackgrass, bermudagrass, johnsongrass, other perennial and annual grasses, cattails, and rushes. It is often used to control established perennial grasses in noncrop areas and along ditchbanks in the western states and as a preplant treatment in crop areas (Meister Publishing Company 1981).

Dalapon has two distinct physiological effects on plants. At high rates of application, dalapon acts as an acid and as a protein precipitant and exerts immediate and localized acute toxic effects. At lower rates of application, dalapon acts slowly as a growth inhibitor (Foy 1975). Dalapon is readily absorbed through both plant roots and foliage. Once absorbed, it is readily translocated throughout the plant tissue, accumulating particularly in regions of highest metabolic activity (Foy 1975; Ghassemi et al. 1981).

Forestry use of dalapon is primarily restricted to ground application for site preparation, conifer release, rights-of-way maintenance, and grass control, as well as to aerial spraying for site preparation and conifer release. Application rates in ground applications for forest management generally range from 0.4 lb. a.i. per acre (0.45 kg/ha), applied around individual trees for conifer release, to an average of 6.8 lb/acre (7.6 kg/ha) for grass control. In aerial spray applications, average dalapon application rates have ranged from 5.0 lb/acre (5.6 kg/ha) for site preparation to 8.6 lb/acre (9.6 kg/ha) for conifer release (Gross 1982). According to product literature summarized by the Weed Science Society of America (1983), dalapon is usually applied at rates ranging from 0.75 lb/acre (0.84 kg/ha) in flax to 20 lb/acre (22.4 kg/ha) or

*Trade names are used only to provide information and do not imply product endorsement. Dowpon® and Radapon® are registered trade names of the Dow Chemical Company. Dalapon is a registered trade name of the Dow Chemical Company abroad.

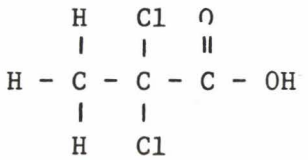
more on noncropland, although under certain conditions multiple treatments at lower rates are used. Statistics on use of dalapon by the USDA Forest Service are presented in Table 1-1.

Dalapon is manufactured in the United States by the Dow Chemical Company and marketed by Vertac Chemical Corporation under the trade name Dowpon®M. Dalapon has also been formulated with other herbicides such as 2,4-D. Table 1-2 lists the major formulations of dalapon and its salts.

Dalapon is normally applied in the spring for site preparation or conifer release. Since dalapon is more toxic to conifers than atrazine, another grass-specific herbicide, atrazine can be added to dalapon when treating Douglas fir plantations to maintain efficacy while reducing overall toxicity to the fir (Newton and Overton 1973 in Newton and Dost 1981). When used for applications other than those involving conifer plantations, 2,4-D may be mixed with Dalapon (as in the formulation Chloropon®) to increase effectiveness on broadleaved weeds (Newton and Dost 1981).

1.2 Physical and Chemical Properties

The active ingredient of dalapon and its formulations is 2,2-dichloropropionic acid with the following structural formula (Weed Science Society of America 1983):



Dalapon is formulated as the sodium and magnesium salts of 2,2-dichloropropionic acid. The major physical and chemical parameters of dalapon and its formulated salts, dalapon sodium salt and dalapon magnesium salt, are detailed in Table 1-3. In general, dalapon and its salts are very soluble in water but have a low solubility in most organic solvents. The partitioning coefficient of dalapon in n-octanol/water is 5.7/1.0 and of dalapon sodium salt is $<1/1.0$ (Kenaga 1974 and Weed Science Society of America 1983). This high water solubility coupled with a low solubility in organic solvents results in a relatively minimal accumulation in tissues of animals exposed to dalapon (See Section 3.1.2).

Dalapon does not absorb to soil particles or to soil colloids to any great extent. This lack of adsorption, coupled with high water solubility, results in a high mobility for dalapon residues that are not decomposed, accumulated, or otherwise bound-up in plants (Foy 1975).

Table 1-1

USDA Forest Service Dalapon Use

Purpose	Active Ingredient Per Acre (lbs.) Range	Average	Acres Treated	Formulation	Application Method
1981					
Aerial					
Conifer release	8.6	8.6	120	Soluble powder applied in a water spray	Aerial, Helicopter
Site preparation	3.7 - 5.7	5.0	778		
Ground					
Conifer release	0.4 - 0.7	0.4	769		Individual tree
Grass control	6.0 - 8.0	6.8	32		Broadcast
Right-of-way	1.3	1.3	273		Broadcast
Site preparation	0.6 - 5.7	2.8	2,286		Spot and Broadcast
1982					
Aerial (2 projects)					
Timber Management	4.0 - 6.0	5.0	53	Soluble powder applied in a water spray	Aerial, Helicopter
Ground (29 projects)					
Timber, Nursery and Wildlife Management, Research, Recreation	1.2 - 30.0	6.7	6,187		Gound foliar

Source: Gross 1982 and 1983.

Table 1-2

Dalapon Formulations

Formulation[a]	Active Ingredients	Formulator
Dowpon Grass Killer[b]	Sodium 2,2-dichloropropionate 85%	Dow Chemical Company
Dowpon® C[b]	2,2-Dichloropropionic acid, sodium salt (dalapon [2,2-dichloropropionic acid] equivalent 46.2%) 53.3% trichloroacetic acid, sodium salt (trichloroacetic acid equivalent 26.2%) 29.7%	Dow Chemical Company
Dowpon® M	2,2-Dichloropropionic acid, sodium salt 72.5%; 2,2-dichloropropionic acid, magnesium salt 12.0%; inert ingredients 15.5%	Dow Chemical Company

[a]Formulations with other herbicides are omitted.
[b]These products have been discontinued.

Source: Gosselin et al. 1976.

Table 1-3

Physical and Chemical Parameters of
Dalapon and Its Formulated Salts

Parameter	Chemical		
	Dalapon	Dalapon Sodium Salt	Dalapon Magnesium Salt
Molecular Formula	$C_3H_4Cl_2O_2$	$C_3M_3Cl_2NaO_2$	$C_6H_6Cl_4MgO_4$
Molecular Weight	143	165	308.5
Physical State (room temperature and pressure)	Liquid	Solid	Solid
Color	Colorless	White	White-tan
Density	1.389 (at 73°F [22.8°C])	--[a]	62.5 lb/cubic foot
Melting Point	--	Decomposes before melting at 379.4 to 386.6°F (193 to 197°C)	Decomposes before melting at 379.4°F (193°C)
Boiling Point	365 to 374°F (185 to 190°C)	--	--
Solubility (g/100g solvent at 25°C) in:			
Water	>80.0	90.0	>80.0
Acetone	--	0.14[b]	--
Methanol	18.5	17.9[b]	35.9
Ethanol	82.7	<18.5	25.5
Benzene	--	0.002[b]	4.4
Ether	Soluble	0.016[b]	--
Kerosene	--	--	0.055

[a]Data not applicable or not available.
[b]Data from Weed Science Society of America, 1970. Herbicide Handbook, WSSA Monograph 3, 2nd ed. Humphrey Press, Geneva, N.Y. All other data are from the Dow Chemical Company.

Source: Kenaga 1974.

Dalapon in aqueous solution is chemically degraded by hydrolysis and by photolytic processes. Metabolic degradation of dalapon and its salts by plants, animals, and microorganisms is discussed in Section 3 of this report. Hydrolysis of dalapon results in the formation of inorganic chlorine compounds (such as sodium chloride) and pyruvic acid, which is the major metabolite of dalapon and its salts. Pyruvic acid is a common naturally occurring organic molecule that is an essential component of energy metabolism (respiration) in living cells. Chemical hydrolysis of solutions of dalapon and its salts is accelerated at alkaline (above 7) pH values. The chemical hydrolytic half-life of dalapon and its salts is probably on the order of several months at temperatures of less than 77°F (25°C) and solutions of less than 1 percent (a 1 percent solution of dalapon sodium salt would have a pH of 5.7, which is acidic) (Kenaga 1974). Aqueous solutions of dalapon also undergo significant rates of photodegradation to form pyruvic acid. Pyruvic acid is then further degraded, primarily to carbon dioxide and acetaldehyde. The acetaldehyde in turn is degraded to acetic acid which then degrades into carbon dioxide and water (Kenaga 1974).

Dalapon is nonflammable. Formulations of dalapon are mildly corrosive to application equipment which should be rinsed with water after use (Weed Science Society of America 1983).

The acid form of dalapon is relatively volatile, and at room temperature, dalapon acid will volatilize rapidly from an aluminum surface. The sodium salt of dalapon, however, volatilizes at negligible rates under similar circumstances (Foy 1975). Since the sodium salt of dalapon is the major constituent of most applications, volatilization of dalapon is not expected to be a significant route of transport from the site of application.

2.0 Toxicity of Dalapon

The toxicological properties of dalapon, summarized in Table 2-1, show that this herbicide is generally nontoxic to a variety of organisms. Dalapon is particularly useful for agricultural crop uses due to its low toxicity to most animal species coupled with its high selectivity to grasses.

2.1 Invertebrates and Microorganisms

A variety of invertebrates (mostly arthropods) and microorganisms from freshwater, marine, and terrestrial ecosystems have been studied for toxic effects of dalapon formulations. Major findings of these studies are presented in Appendix A and indicate that, with the exception of several studies in aquatic crustacea and insects in which toxic effects were reported, dalapon should be considered only slightly toxic to most invertebrates and microorganisms. Many studies cited in Appendix A indicate median lethal concentrations of dalapon in excess of 100 ppm, and several indicate median lethal concentrations in excess of 1,000 ppm.

For the most part, toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of dalapon in a natural environment. For example, in the laboratory, aquatic organisms were generally kept up to 4 days or more in static conditions (in tanks or aquaria without a flow-through of freshwater). Microorganisms were generally grown in culture media containing levels of dalapon higher than those resulting from normal application. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to dalapon or to other man-made chemicals is usually a transient phenomenon depending on stream flow, chemical dissipation, or decomposition. Additionally, in natural systems, interactions with other man-made and naturally occurring chemicals and changes in environmental factors potentially affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions, in turn, potentially alter tolerance levels of organisms to the herbicides.

Among the insect studies summarized in Appendix A, those with honeybees, representing insects of commercial importance, indicate that dalapon is relatively nontoxic. In studies by the Dow Chemical Company reported by Kenaga (1974), honeybees momentarily dipped in a solution containing 20,000 ppm dalapon showed no mortality 24 hours later. In ingestion studies, an increased mortality was observed in bees fed 1,000 ppm dalapon by weight in sucrose, but no increased mortality compared to controls was observed in bees fed 100 ppm dalapon or less (Morton et al. 1972). Other researchers (Atkins and

Table 2-1

Summary of Toxicological Properties of Dalapon[a]

1. Acute Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (male) (Dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 9,330 (8,460-10,300) mg/kg
Rat (female) (Dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 7,570 (6,880-8,350) mg/kg
Mouse (female) (Dalapon sodium salt)	Ingestion of a single dose	LD_{50} >4,600 mg/kg
Guinea pig (female) (Dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 3,860 (2,760-5,430) mg/kg
Rabbit (female) (Dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 3,860 (2,300-6,500) mg/kg
Rat (male & female) (Dowpon®: 85% dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 5,660 mg/kg
Guinea pig (female) (Dowpon®: 85% dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 4,930 mg/kg
Rabbit (female) (Dowpon®: 85% dalapon sodium salt)	Ingestion of a single dose	LD_{50} = 2,830 mg/kg
Rat (female) (Dowpon® M: 72.5% dalapon sodium salt and 12% dalapon magnesium salt)	Ingestion of a single dose	LD_{50} = 5,660 mg/kg
Guinea pig (female) Dowpon® M (72.5% dalapon sodium salt and 12% dalapon magnesium salt)	Ingestion of a single dose	LD_{50} = 2,830 mg/kg
Rabbit (female) Dowpon® M (72.5% dalapon sodium salt and 12% dalapon magnesium salt)	Ingestion of a single dose	LD_{50} = 2,140 mg/kg

Table 2-1 (Continued)

1. Acute Oral Toxicity (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Fish (85% dalapon sodium salt)	Surface absorption	LC_{50} >100 ppm
Birds (Formulation not specified)	5-day ingestion	No mortality at 5,000 ppm in feed.

2. Primary Eye Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (Dalapon sodium salt)	Instillation of dry powder.	Pain, irritation of conjunctivae, followed by severe conjunctivitis and corneal injury which healed after several days.
Rabbit (Dalapon sodium salt)	Instillation of 10% solution.	Slight pain and conjunctivitis.
Rabbit (Dalapon sodium salt)	Instillation of 1% solution.	No reaction.

3. Subchronic Oral Toxicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Cattle (Dalapon sodium salt)	500 mg/kg for 10 days by capsule	None
Cattle (Dalapon sodium salt)	250 mg/kg for 10 days, 500 mg/kg for 9 days by drench	Throat irritation at 500 mg/kg.
Sheep (Dalapon sodium salt)	50 and 100 mg/kg for 10 days by drench	None
Rat (Dalapon sodium salt)	115 to 1,150 mg/kg/body wt in diet for 97 days	No deaths observed. At high concentrations, there was some growth retardation, and increase in average weight of liver and kidneys which showed slight histopathological changes.

Table 2-1 (Continued)

3. Subchronic Oral Toxicity (Concluded)

Organism (Formulation)	Exposure	Adverse Effects
Sheep (Dalapon sodium salt)	100 mg/kg/day for 35 days	None
Sheep (Dalapon sodium salt)	100 mg/kg for 481 days by capsule	Weight loss of 10% after 86 treatments.

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Calf (female) (Dalapon sodium salt)	1,000 mg/kg/day by intubation	General lassitude, diarrhea, loss of apetite, slight loss of weight, slowed pulse, discharge from eyes, and mild cyanoses of mucous membrane. Recovered completely 4 days following last dose.
Calf (male) (Dalapon sodium salt)	1,000 mg/kg/day by intubation	Showed only slight kidney involvement, e.g. swelling of cells.
Calves (Dalapon sodium salt)	Fed on oats treated with 2.5 to 20 lb/acre	None
Calves (Dalapon sodium salt)	Fed on grassy pasture treated with 30 lb/acre; sudan grass treated with 20 lb/acre	None
Sheep (Dalapon sodium salt)	Fed on oats treated with 2.5 to 20 lb/acre	None
Hogs, sheep, cattle (Dalapon sodium salt)	12,500 ppm in drinking water for 2 weeks	None
Dog (Dalapon sodium salt)	Given by capsule 5 times weekly, for 2 weeks, then adjusted upward weekly to a maximum of 1,000 mg/kg over an 80 day period.	Except for vomiting, there were no other signs of toxicity.

Table 2-1 (Continued)

4. Subchronic Dermal Toxicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rabbit (Dalapon sodium salt)	Undiluted, 10% or 1% solution applied to intact or abraded skin as 10 applications in 14 days	Moderate hyperemia and slight superficial necrosis after 3 or 4 applications of undiluted material to intact skin. Complete healing 1 week following final application; 10% solution only slightly irritating; no adverse effects with 1% solution.

5. Chronic Toxicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Dog (Dalapon sodium salt)	15, 50 or 100 mg/kg by capsule 5 days weekly for 52 weeks	Increase in average kidney weight in animals receiving 100 mg/kg/day. No other toxic signs.
Rat (Dalapon sodium salt)	Fed in diet at 5, 15, and 50 mg/kg/day for 2 years	Increase in average kidney weights in animals receiving 50 mg/kg/day.

6. Carcinogenicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Dalapon sodium salt)	Fed in diet at 5, 15, and 50 mg/kg/day for 2 years	None

Table 2-1 (Concluded)

7. Reproductive and Teratogenic Effects

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Dalapon)	250 to 2,000 mg/kg/day administered to pregnant animals from day 6 through 15 of gestation	No teratogenic effects. Fetal resorption rate and lower weight gains of dams at 1,500 and 2,000 mg/kg. NOEL was 1,000 mg/kg.
Rat (Dalapon)	500 to 1,500 mg/kg/day administered from day 6 through 15 of gestation	No major dose related abnormalities reported. Reduced weights of pups at 1,000 and 1,500 mg/kg/day dosage levels.
Rat (Dalapon sodium salt)	30 to 300 mg/kg/day in diet for three generations	No adverse effect on fertility, gestation, lactation, viability or normal growth and maturation.

8. Mutagenicity

The mutagenic potential of dalapon cannot be evaluated since no mutagenicity tests have been reported in the literature.

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

Anderson 1967 and Duval 1969, both in Kenaga 1974) have also found dalapon toxicity in honeybees to be low. However, King (1964) claimed, without supporting data, that dalapon has a very high contact toxicity to honeybees and concluded that dalapon should not be used in areas of high bee activity. The lack of data supporting King's conclusions, and studies since 1964 indicating relative nontoxicity of dalapon to honeybees, cast doubt on the claim for contact toxicity of dalapon to bees.

Other insect studies with dalapon summarized in Appendix A report dalapon (sodium salt) toxicity ranging from a 24-hour LC_{50} of 1.0 ppm for stonefly nymphs (Cope 1965 in Pimentel 1971) to a 48-hour LC_{50} of 1,600 ppm (a.e.) for dragonflies. In this latter study, no mortality was observed (Dow Chemical Company data reported by Kenaga 1974). It should be noted that the high toxicity of dalapon to stoneflies reported by Cope in 1965 is contradicted by more recent studies in which the median lethal toxicity of dalapon to stonefly nymphs was reported to be greater than 100 ppm in a 96-hour flow-through bioassay with no toxic effect observed at the 100 ppm concentration (Sanders and Cope 1968 in Folmar 1977). Johnson and Finley (1980) also found no toxicity at concentrations of more than 1,000 ppm in a 96-hour test with 75.6% a.i. material.

A number of studies have been conducted with both marine and freshwater crustaceans that show a wide toxic response in these animals to dalapon, ranging from a 48-hour EC_{40}* of 1.0 ppm (87% a.i.) in brown shrimp (Butler 1965) to a 96-hour LC_{50} of 4,800 ppm (73.5% a.i.) in a harpacticoid crustacean (an important component of zooplanktonic food supply in brackish waters) (Linden et al. 1979). In general, dalapon is more toxic to cladocerans, such as water fleas (Daphnia), with median lethal toxicities ranging from 6 ppm in a 48-hour test (U.S. Department of the Interior 1968 in Pimentel 1971) to 16 ppm in a 48-hour test (Johnson and Finley 1980), than to most invertebrates tested. The median lethal concentration of dalapon to shrimp of the genus Crangon was greater than 100 ppm in a 24-hour static bioassay (Portmann and Wilson 1971 in Folmar 1977). Other crustaceans studied were able to tolerate a 24-hour immersion in 200 ppm (a.e.) dalapon with no mortality (Dow Chemical Company data cited in Kenaga 1974).

*EC_{40}: 40 percent of the test animals died or were paralysed.

In studies with molluscs, dalapon was only slightly toxic. Oysters in a 96-hour flow-through bioassay with 1.0 ppm dalapon showed no toxic effect and no reduced rate of shell growth (Butler 1965). In a test with cockles (a clam-like bivalve mollusc), Portmann and Wilson (1971 in Folmar 1977) found that the median lethal concentration of dalapon was greater than 100 ppm. In snails, 50 percent mortality occurred at concentrations of 400 ppm a.e. of dalapon, but at 200 ppm there was no mortality (Dow Chemical Company data cited in Kenaga 1974).

Various studies with soil organisms (arthropods, roundworms and segmented worms) and microorganisms presented in Appendix A indicated a generally low toxicity of dalapon to the different species tested. Courtney et al. (1962 in Pimentel 1971) found a 94 percent reduction in nematode populations in a field situation when dalapon was applied at a rate equivalent to 5 lb/acre (5.6 kg/ha). On the other hand, Fox (1964 in Kenaga 1974), in a field application of dalapon at a rate equivalent to 20 lb/acre (22.4 kg/ha), found an increase in populations of soil arthropods and no change in earthworm populations. Soil microorganisms were unaffected by as much as 26 ppm dalapon incorporated into soil samples and incubated in the laboratory for 32 weeks, but at concentrations of 266 ppm dalapon and more, nitrification was inhibited (Greaves et al. 1981). Studies with several species of Rhizobium, a soil bacterium that is associated with nitrogen fixation by legumes, demonstrated that some strains were sensitive to dalapon at concentrations in culture media of 72.6 ppb (Faizah et al. 1980); however, at least one strain was capable of using dalapon as its sole source of carbon and energy (Berry et al. 1979). In a calcareous loam soil treated with dalapon at a concentration equivalent to an application rate of 5 tons/acre, carbon dioxide evolution by soil microbial populations was reduced by 21 percent compared to controls (Stojanovic et al. 1972).

Studies with algae, summarized in Appendix A, indicate that dalapon is slightly toxic to most species as tested. Cullimore (1975) found that some strains of algae showed reduced growth at dalapon concentrations up to 10 ppm. Five species of blue-green algae tolerated exposure for 15 days to dalapon doses equivalent to application rates of 179 lb/acre (200 kg/ha) (Venkataraman and Rajyalakshmi 1971 in Kenaga 1974). Marine unicellular algae cultured in test tubes with dalapon and artificial seawater had LC_{50} values of 20 to 100 ppm for a 10-day exposure. When formulated Dowpon® M was incorporated in the growth media for these same species of algae in place of dalapon, LC_{50}'s of 400 to 500 ppm were observed and less inhibition of oxygen production (by a factor of 100) occurred (Walsh 1972 in Kenaga 1974).

2.2 Fish and Amphibians

The toxic effects of dalapon and its sodium salt on fish and amphibians have been reported in numerous studies summarized in Appendix B. Dalapon is only slightly toxic to fish and amphibians. Reported toxicities range from a 48-hour LC_{50} of 44 ppm in the harlequin fish (Alabaster 1969 in Folmar 1977) to a 96-hour LC_{50} in excess of 30,000 ppm (no mortality was observed) in the grass carp (Tooby et al. 1980). Fish toxicity studies with dalapon and its sodium salt formulations were reviewed by Kenaga (1974), who had access to Dow Chemical Company documentation. Representative data are summarized, by fish species, in Table 2-2 and indicates LC_{50}'s greater than 100 ppm for all species tested with exposures ranging from 24 hours to 21 days. Clark et al. (1970) suggest that pesticides with median lethal concentrations of 10 ppm or more for fish be considered "slightly toxic."

At the high doses of dalapon that fish and amphibians have been subjected to in the studies described in Appendix B, however, several sublethal effects have been observed. Rainbow trout fry showed an avoidance response to dalapon at concentrations of 1.0 and 10.0 ppm but were unresponsive to dalapon concentrations of 0.1 ppm (Folmar 1976). Johnson (1976) demonstrated that 96-hour exposure to 4,000 ppm dalapon reduced the temperature tolerance of frog tadpoles compared to controls.

2.3 Birds

Dietary studies and egg injection bioassays with dalapon indicate a low toxicity to birds (Appendix C). Japanese quail, ring-necked pheasant, and mallard ducks ingested up to 5,000 ppm dalapon in their diet for 5 days without mortality according to Hill et al. (1975). In other studies reviewed by Kenaga (1974; U.S. Department of the Interior 1963, 1964; and Dewitt 1962), pheasants, bobwhite, and mallards all suffered some mortality at dalapon concentrations of 5,000 ppm in food during chronic feeding studies (100 to 169 days) and mallards had high levels of mortality with dalapon concentrations of 1,000 ppm in food. In chickens, Paynter et al. (1960 in Kenaga 1974) determined an acute oral LD_{50} of 5,660 mg/kg body weight for mixed sexes of chicks. Palmer and Radeleff (1969) reported that after 10 days of ingestion by capsule, no effects were seen in chickens at 100 mg/kg body weight, but at 250 and 500 mg/kg body weight, reduced weight gain compared to controls was observed.

Table 2-2

Studies of Fish Toxicity Studies with Dalapon Formulations

Fish	Exposure Time	Effects	Comments	References[a]
Rainbow trout	24 hours to 21 days	LC_{50} from >100 to 430 ppm for salt formulation (from >87 to 318 ppm dalapon content). In one test (Reference B), no mortality was observed after 96 hours at 50°F in 300 ppm salt formulation (222 ppm dalapon).	Temperatures 50 to 68°F (10 to 20°C). Formulations included technical dalapon, Dowpon® and Dowpon®S.	A, B, H
Brown trout	24 to 96 hours	LC_{50} = 345 ppm salt formulation (255 ppm dalapon content). LC_{100} = 500 ppm salt formulation (370 ppm dalapon) after 72 hours. No mortality at 300 ppm salt formulation (222 ppm dalapon) after 96 hours.	Temperature = 50°F (10°C). Formulation used was Dowpon®S.	B, J
Coho salmon	24 to 96 hours	LC_{50} from 310 to 340 ppm for sodium salt formulation (269 to 295 ppm dalapon).	Temperature = 68°F (20°C). Formulation used was Dowpon®S.	C
Bluegill	24 hours to 21 days	LC_{50} from >100 to 510 ppm for sodium salt formulation (from >87 to 442 ppm dalapon content). No mortality was observed after 7 days in 80 ppm technical dalapon (Reference E) or after 21 days in 50 ppm technical dalapon (Reference F)	Temperatures from 54 to 77°F (12 to 25°C). Formulations included technical dalapon and Radapon®.	E, F, G, I
Yellow perch	72 hours	No mortality at 300 ppm for salt fromulation (222 ppm dalapon), 100% mortality at 400 ppm for salt formulation (296 ppm dalapon).	Temperature = 75°F (24°C). Formulation used was Dowpon®S.	J
Pumpkinseed	72 hours	No mortality at 400 ppm for salt formulation (296 ppm dalapon), 75% mortality at 500 ppm salt formulation (370 ppm dalapon).	Temperature = 75°F (24°C). Formulation used was Dowpon®S.	J

Table 2-2 (Continued)

Fish	Exposure Time	Effects	Comments	References[a]
Largemouth bass	48 hours to 7 days	100% mortality in flow-through bioassay with 1,000 ppm sodium salt formulation (867 ppm dalapon) at 7 days. No mortality in static bioassay with 1,000 ppm sodium salt formulation (867 ppm dalapon) at 48 hours or with 250 ppm sodium salt formulation (217 ppm dalapon) for 7 days in flow-through bioassay.	Temperature = 68°F (20°C). Formulation used was Dowpon®.	C
Channel catfish	96 hours	LC_{50} = >100 ppm for sodium salt formulation (>87 ppm dalapon).	Temperature = 50°F (10°C). Technical dalapon formulation used.	H
Goldfish	48 to 96 hours	LC_{50} = >100 ppm for sodium salt formulation (>85 ppm dalapon) at 96 hours (Reference H). No mortality was reported at concentrations as high as 200 ppm dalapon for 96 hours by the other two references.	Temperatures from 50 to 83°F (10 to 28°C). Technical dalapon, Dowpon®, and Dowpon® M formulations used.	H, D, E
Fathead minnow	24 to 96 hours	LC_{50} values from 290 to 480 ppm salt formulations (251 to 355 ppm dalapon). No mortality reported at 200 and 300 ppm formulation (148 and 222 ppm dalapon). 100% mortality was reported for 500 ppm salt formulation (370 ppm dalapon) after 72 hours.	Temperature from 50 to 77°F (10 to 25°C). Dowpon®, Dowpon® S and Radapon® formulations used. All else being equal, toxicities were greater when measured in salt water than in hard (Reference G).	B, J, G
Lake Emerald shiner	72 hours	No mortality at 4,000 ppm sodium salt formulation (2,960 ppm dalapon).	Temperature = 57°F (14°C). Formulation used was Dowpon®.	J
Harlequin fish	24 to 48 hours	LC_{50} from 240 to 300 ppm salt formulation (178 to 222 ppm dalapon).	Temperature = 68°F (20°C). 20% replacement of treated water every 10 minutes. Formulation used was Dowpon®.	A

Table 2-2 (Concluded)

[a]References cited in Kenaga 1974:

A Alabaster, J.S., 1969. "Survival of Fish in 164 Herbicides, Insecticides, Fungicides, Wetting Agents and Miscellaneous Substances." Int. Pest. Control 2:29-35.

B Alexander, H.C., 1963. Toxicity of Dowpon® S to Three Species of Fish. Letter to G.E. Lynn, The Dow Chemical Company, Midland, Michigan, September 10, 1963.

C Bond, C.E., R.H. Lewis and J.L. Fryer, 1960. Toxicity of Various Herbicidal Materials to Fishes. Transactions 1959 Seminar: Biological Problems in Water Pollution. The Robert A. Taft Engineering Center, Technical Rept. W60-3. Public Health Service, U.S. Department of Health, Education and Welfare, Cincinnati, Ohio.

D Hardy, J.L., 1971. Effect of Dowpon® M on Goldfish. Letter to F. Hoerger, The Dow Chemical Company Midland, Michigan, July 29, 1971.

E Lawrence, J.M., 1962a. Aquatic Herbicide Data. Agricultural Handbook No. 231, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

F Lawrence, J.M., 1962b. Aquatic Herbicide Data, Supplement I. Agricultural Experiment Station, Auburn University, Auburn, Alabama.

G Surber, E.W. and Q.H. Pickering, 1962. Acute Toxicity of Endothal, Diquat, Hyamine, Dalapon and Silvex to Fish. The Progressive Fish-Culturist 24(4):164-171.

H Svendsen, G.E., 1966. Dalapon, Sodium Salt in Acute Toxicity Studies. Letter to Ralph L. Gantz, The Dow Chemical Company, March 11, 1966.

I U.S. Department of the Interior, 1965. Pesticide-Wildlife Studies: Effects of Pesticides on Fish and Wildlife 1964, Circular 226. Fish and Wildlife Service, U.S. Department of the Interior, Washington, D.C.

J Winston, A.W., Jr., 1964. Fish Toxicity of Dalapon and Dowpon®. Letter Report to G.E. Lynn, Waste Control Laboratory, The Dow Chemical Company, Midland, Michigan. September 18, 1964.

Reproductive effects have been reported when dalapon was administered at high doses to birds. When mallards were fed dalapon at levels that were less than 25 percent of doses which produced mortality, reproduction was depressed (U.S. Department of the Interior 1962 in Pimentel 1971). Dunachie and Fletcher (1970) reported a decreased rate of hatching compared to controls when eggs were injected with dalapon at doses giving 300 ppm in the egg, but not at doses equivalent to 200 ppm. No teratogenic effects were observed in these tests, however. When female bobwhites were fed toxic lethal doses (1,000 to 5,000 ppm in diet), they did not lay fewer eggs than controls, but similarly dosed mallards and pheasants did lay fewer eggs. Those eggs actually laid by treated females did not show decreased viability or hatching success compared to eggs laid by control females (U.S. Department of the Interior 1963 and Dewitt 1962 in Kenaga 1974).

2.4 Mammals

The potential health hazards from exposure to dalapon have been evaluated in laboratory animals such as mice, rats, guinea pigs, rabbits and dogs as in well as in domestic animals such as cattle, sheep and hogs. The results of the various toxicity tests are described below and are summarized in Table 2-1 and in Appendix D.

2.4.1 Acute Toxicity

Acute toxicity data have been reported for oral and eye irritation studies.

2.4.1.1 Oral. Dalapon sodium salt and dalapon formulations are very low in acute toxicity to all mammals tested as indicated by the high LD_{50} values (Paynter et al. 1960 and Kenaga 1974). The oral LD_{50} for dalapon sodium ranged from 9,300 mg/kg in rats to 3,860 mg/kg in guinea pigs and rabbits. In mice, the LD_{50} for dalapon sodium salt was greater than 4,600 mg/kg. The acute oral LD_{50} for Dowpon® grass killer ranged from 5,660 mg/kg in rats to 2,830 mg/kg in rabbits. Acute oral LD_{50} values of 4,930 mg/kg were obtained with guinea pigs in a study with Dowpon® grass killer. For Dowpon® M grass killer, the acute LD_{50} ranged from 5,660 mg/kg in rats to 2,140 mg/kg in rabbits, with an intermediate LD_{50} of 2,830 mg/kg in guinea pigs. According to U.S. Environmental Protection Agency toxicology guidelines these toxicity ranges place dalapon into toxicity categories III and IV (See Table 2-1 of the General Introduction to Herbicide Background Statement).

2.4.1.2 Primary Eye Irritation. Dalapon is moderately irritating to eyes (Paynter et al. 1960). When dry, powdered dalapon sodium salt was placed into the eye of a rabbit, the immediate response was slight irritation of the conjunctival membranes. This

was followed by severe conjunctivitis and corneal injury which healed completely after several days. Washing of the eye with copious amounts of water immediately after instillation of the material greatly reduced the severity of irritation produced. A 10 percent aqueous solution of the sodium salt caused only very slight conjunctivitis and a 1 percent solution produced no adverse reactions whatsoever.

2.4.2 Subchronic Toxicity

Subchronic toxicity data have been reported for oral and dermal studies.

2.4.2.1 Oral. Very few toxic signs were observed when animals were treated with repeated doses of dalapon sodium salt. In a study reported by Paynter et al. (1960), rats were maintained on diets containing 0.0115 to 1.15 percent (equivalent to 11.5 to 1,150 mg/kg/day) dalapon sodium salt for 97 days. No deaths occurred, and in male rats there were no adverse effects at levels of 0.115 percent or below. In female rats, there were slight increases in average kidney weights at the 0.0346 and 0.115 percent dietary levels, but no other gross or histopathological effects that differed from those seen in control animals. At the higher doses of 0.346 and 1.15 percent (equivalent to 346 and 1,150 mg/kg/day), findings of significance were limited to growth retardation, increases in average weight of liver and kidneys, and minor unspecified histopathological changes in liver and kidneys. No other evidence of any adverse effects were determined by hematological examination, measurement of average body weights, or microscopic examination of the lungs, heart, and spleen of treated animals.

Two mongrel dogs were given dalapon sodium salt by capsule 5 days a week over an 80-day period. The animals received 50 mg/kg/day for the first 2 weeks. The dosages were adjusted upward weekly thereafter until a maximum dose of 1,000 mg/kg/day was reached. Except for vomiting, the dogs exhibited no other signs of systemic toxicity as revealed by hematological and biochemical studies, urinalyses, or autopsy of organs and tissues (Paynter et al. 1960).

No signs of toxicity were observed when dalapon sodium salt was administered to cattle by capsule (500 mg/kg) for 10 days. When administered daily to cattle by oral drench (250 mg/kg for 10 days; 500 mg/kg for 9 days), the only adverse effect observed was throat irritation in animals receiving the 500 mg/kg dose. No signs of toxicity were observed when dalapon sodium salt was administered to sheep by oral drench (50 and 100 mg/kg) for 10 days (Kenaga 1974).

When dalapon sodium salt was administered to sheep by oral capsule (100 mg/kg) for 10 days, animals became ill and showed a 6 percent weight loss. Sheep treated with 100 mg/kg/day for 481 days exhibited a 10 percent weight loss after 86 treatments (Kenaga 1974).

Paynter et al. (1960) treated a heifer and a bull calf daily for 10 days by oral intubation of 1,000 mg dalapon sodium salt per kg body weight. The only sign of toxicity exhibited in the male calf was slight kidney involvement, revealed by histological examination as swelling of the convoluted tubules and hypertrophy of the glandular cells. The female calf exhibited general lassitude, diarrhea, coat roughness, loss of appetite, slight loss of weight, slowed pulse rate, discharge from the eyes, and mild cyanosis of mucous membranes. The animal recovered completely 4 days following the last dose. Paynter et al. (1960) also reported that dosing of sheep and cattle with dalapon sodium salt at 100 mg/kg/day for 35 days produced no adverse effects.

Goldstein and Long (1960 in Kenaga 1974) reported that no adverse effects were observed when calves were allowed to graze on grassy pasture treated with dalapon sodium salt at 30 lb/acre or when calves or sheep were fed on oats treated with the chemical at 2.5 to 20 lb/acre. The authors reported that calves grazed on the treated pasture until vegetation turned brown, otherwise, the exposure durations in these field studies were not reported.

When hogs, sheep and cattle were allowed to drink water containing 12,500 ppm dalapon sodium salt for 2 weeks, no adverse effects were noted (Goldstein and Long 1960 in Kenaga 1974).

2.4.2.2 Dermal Irritation. Paynter et al. (1960) applied undiluted dalapon sodium salt or either 10 percent or 1 percent aqueous solutions of the salt to intact or abraded skin of the shaved abdomen of rabbits. The animals were given 10 continuous applications over a 10-day period. The authors reported that the undiluted material produced moderate hyperemia and slight superficial necrosis of intact skin after 3 or 4 applications. The injured skin healed without scarring within 1 week after final application. Prolonged contact of the herbicide abraded skin produced necrosis. A 10 percent solution was only slightly irritating and a 1 percent solution caused no irritation to either intact or abraded skin.

There was no evidence that dalapon sodium salt was absorbed through the skin in toxic amounts (Weed Science Society of America 1983).

2.4.3 Chronic Toxicity

Paynter et al. (1960) administered dalapon sodium salt by capsule (15, 50, or 100 mg/kg/day) to dogs 5 days a week for 52 weeks. At the two lower doses, the dogs exhibited normal behavior, gained weight, and showed no adverse effects. In animals receiving 100 mg/kg/day, adverse effects were limited to an increase in average kidney weight. Histopathological examination revealed no significant difference in tissues between treated and untreated control animals.

Rats were fed diets containing 100, 300, and 1,000 ppm dalapon sodium salt, equivalent to 5, 15 and 50 mg/kg/day, for 2 years. Adverse effects were limited to increases in average kidney weights in rats treated with 50 mg/kg/day. Microscopic examination of tissues revealed no abnormal pathology.

2.4.4 Reproductive and Teratogenic Effects

In a multigeneration reproduction study (Paynter et al. 1960), rats were fed diets containing 0.03, 0.1 or 0.3 percent dalapon sodium salt for three generations. This is equivalent to approximately 30, 100, and 300 mg/kg/day. No adverse effects on fertility, gestation, viability, or normal growth and maturation were observed.

In a teratogenicity study in rats, dalapon was administered daily by gavage at doses of 250, 500, 1,000, 1,500, or 2,000 mg/kg to pregnant animals from the 6th through 15th day of gestation (Thompson et al. 1971 in Kenaga 1974). No teratogenic effects were noted. However, at the 1,500 and 2,000 mg/kg dosage levels the rate of fetal resorption was increased but not significantly different from controls and weight gains for pregnant dams were lower than controls. Pup weights at the 1,000 mg/kg dosage level were significantly lower than controls. No adverse effects were observed at dosage levels of 1,000 mg/kg day or below.

In another teratogenicity study in rats (Emerson et al. 1971 in Kenaga 1974), dalapon was administered daily by gavage at doses of 500, 1,000 or 1,500 mg/kg/day from the 6th through 15th day of gestation. No major dose related skeletal or visceral abnormalities were observed. At the 1,000 and 1,500 mg/kg/day dosage levels, reduced weights of the offspring were observed.

2.4.5 Carcinogenicity

In a 2-year feeding study (Paynter et al. 1960), groups of 20 to 24 albino rats of the Carworth Farm strain were fed diets containing 100, 300, or 1,000 ppm dalapon sodium salt. This diet was equivalent to approximately 5, 15, and 50 mg/kg/day. Histological examination of tissues from representative animals sacrificed at 104 weeks

revealed no differences compared to control animals. Findings specifically related to tumor formation were not reported.

2.5 Mutagenicity

The mutagenic potential of dalapon or its formulations cannot be evaluated since mutagenicity testing of these materials has not been reported in the literature.

3.0 Environmental Fate

Dalapon is selectively toxic to annual and perennial grasses, yet is toxic to only a few crop species. It has a short persistence in soil, thus exhibiting only temporary effects (Ashton 1982), disappears rapidly from water (Ghassemi et al. 1981), and is only slightly toxic to most animal species (Kenaga 1974). Dalapon is absorbed by both plant roots and foliage and, once absorbed, is readily translocated throughout the plant. It accumulates in those regions of the plant where metabolic activity is high. The available evidence suggests that dalapon is not metabolized in plants and, at least in some species, is extremely persistent with some dalapon carried via seeds through as many as three generations (Foy 1975). In animals, dalapon breaks down by hydrolysis to pyruvate, a normal metabolic intermediate that then further degrades to acetate and carbon dioxide (Foy 1975).

3.1 Bioaccumulation and Metabolism

Bioaccumulation and metabolism of dalapon in both animals and plants were extensively reviewed by Kenaga (1974) and by Foy (1975). The following discussion draws extensively upon these two reviews. In particular, the discussion on animal bioaccumulation and metabolism relies primarily on the review by Kenaga (1974) who in turn relied extensively on published and unpublished reports by Dow Chemical Company, many of which are unavailable otherwise. The specific data are reported in Kenaga (1974).

3.1.1 Plants

The major pathways for the uptake of dalapon by plants are through foliage and roots. Dalapon is readily translocated through the plants once it is absorbed and appears to accumulate most readily in regions of high metabolic activity such as areas of new growth, budding, and fruiting activity (Ghassemi et al. 1981). Short-term studies on the order of two weeks suggest that dalapon is not metabolized by plants, although long-term studies have yielded results that are equivocal due to the dilution of dalapon levels in the plant tissues as a result of plant growth (Foy 1975).

In a series of studies to determine the metabolic pathways of plants affected by various herbicides, Ashton et al. (1977) found that dalapon inhibited RNA synthesis, photosynthesis, lipid synthesis, and protein synthesis in descending order of importance, but did not have any inhibitory effect on plant respiration. At the lowest concentration at which appreciable inhibition was seen in plant cell cultures after 2 hours (10^{-3}M concentration of dalapon), RNA synthesis was inhibited 44 percent, photosynthesis 30 percent, lipid synthesis 20 percent, and protein synthesis 14 percent.

The long-term storage of dalapon in several species was reported in a series of studies summarized by Foy (1975). When wheat seedlings were exposed to preplant applications of dalapon in the field at a rate equivalent to 4 lb/acre (4.48 kg/ha), dalapon was carried by seeds to the third generation.

Kenaga (1974) reviewed data from the Dow Chemical Company on dalapon residues in plants (Gentry and van Middelem 1971; Getzendaner 1964, 1972; and Hoerger and Kenaga 1972). In general, residue levels found in a plant are directly proportional to the rate of application. These residues also decrease with time following application and are a function of plant surface area-to-weight ratio with structures such as leaves, having the highest ratio, also having the highest level of dalapon residues following application. Thus, in grass treated with Dowpon® Grass Killer, residues immediately after application ranged from 135 to 48 ppm dalapon per pound of Dowpon® applied per acre, but 10 weeks after application, these residues had decreased to less than 1 ppm dalapon per pound Dowpon® applied per acre. Grass represents foliage with a high surface area-to-weight ratio. Asparagus, on the other hand, representing stems with a low surface area-to-weight ratio, tend to have lower residues of dalapon, ranging from 0.74 to 1.2 ppm for each pound of Dowpon® applied per acre three days after application to 0.07 ppm for each pound applied per acre 15 days after application.

3.1.2 Animals

Dalapon has a very high solubility in water but is not readily soluble in most organic solvents (Weed Science Society of America 1983). As a result of these differing solubilities, dalapon does not tend to dissolve in the organic constituents of animal cells, yet is readily carried out of tissues and cells by aqueous fluids such as urine or surrounding water in freshwater and marine aquatic organisms. The resulting lack of any strong bioaccumulation of dalapon in animals is indicated by a wide range of studies, mostly reported in material generated by the Dow Chemical Company and summarized in Kenaga (1974). This information is presented in Appendix F.

Dalapon is rapidly excreted in the urine of animals when ingested and very few residues are found in tissues of animals fed dalapon. In a human excretion study, Hoerger (1969 in Kenaga 1974) found that 50 percent of total dalapon was excreted in the urine within 18 days following 5 daily doses of 0.5 mg/kg body weight (amounting to a total dose of 198.3 mg). In several studies with dogs, ranging from single acute oral doses to year-long dietary studies, most of the dalapon ingested was excreted in the urine. The half-life of dalapon in the blood was 12 hours. With a daily dose of 100 mg/kg body weight, maximum residues of 69 ppm were found in the

kidneys (Leasure 1964; Hoerger 1969; and Paynter et al. 1960 all in Kenaga 1974). Cows fed as much as 200 ppm dalapon in the diet for as long as 8 weeks produced milk with up to 1 ppm dalapon within 3 to 5 days of initiation of dosage and then maintained a constant level of residue in milk (Kutschinski 1961 in Kenaga 1974). Milk from cows fed radiolabeled dalapon contained only two radioactive compounds, dalapon and chloride ions, derived from the original dalapon (Redemann and Hamaker 1959 in Kenaga 1974). When steers were fed dalapon at 100 ppm in their diet, the highest residue levels were in the blood (5.9 ppm) and kidney (2.5 ppm) with a dalapon residue half-life in tissues of 2 days when the animals were withdrawn from dalapon in the feed (Getzendaner 1969b in Kenaga 1974). Pigs fed up to 200 ppm dalapon in the diet for as long as 49 days, and rats fed up to 1,000 ppm dalapon in the diet for 2 years, had highest levels of dalapon in the kidneys and lowest residue levels measured in fat (Paynter et al. 1960 and Getzendaner 1969a in Kenaga 1974). When laying hens were fed up to 50 ppm dalapon in their feed for as long as 60 days, maximum dalapon residue levels of 22 ppm were measured in blood and kidneys and residues in eggs averaged 1.4 ppm and never exceeded 2.0 ppm (Getzendaner 1968 in Kenaga 1974).

3.2 Soil

The fate and transport of dalapon in soil have been reviewed extensively by Kenaga (1974) and by Foy (1975). Dalapon has a very short persistence (generally one month or less) in soil compared with other pesticides and thus exhibits only temporary effects on soil biota (Ashton 1982). Dalapon does not adsorb readily to soil particles and this characteristic, coupled with the relatively high solubility of dalapon in water, allows dalapon to be readily leached from soil. The major means of removal of dalapon from soil, however, appears to be by microbial degradation. Chemical degradation and volatilization play a minor role in dalapon loss from soil. Photodegradtion, at least in field situations, appears not to occur with dalapon (Foy 1975; Ghassemi et al. 1981 and Kenaga 1974).

Helling (1971) compared the mobility of 40 pesticides on plates of silty clay loam and found dalapon to be among the most mobile of the pesticides studied. This high mobility is confirmed in a number of studies reviewed by Foy (1975). One factor leading to the high mobility of dalapon is its lack of adsorption to soil particles. In clay and clay-loam soils ranging from 30 to 67 percent clay, the percentage of dalapon adsorbed to particles can be essentially zero, while in a muck soil, 20 percent of applied dalapon can be adsorbed (Kearney et al. 1965 in Foy 1975). In general, the physical interactions between soil particles and dalapon are relatively unimportant in determining leaching of dalapon.

The laboratory studies discussed above suggest that leaching of dalapon from soil should be a major potential route for the loss of herbicidal activity in soils treated with dalapon given favorable precipitation and soil type. However, in actual field studies reviewed by Kenaga (1974), it was found that dalapon had not leached through the soil as would be expected, suggesting that microbial degradation of dalapon occurs more rapidly than leaching under conditions favorable to microbial growth.

A large number of studies have shown that soil microorganisms are capable of degrading dalapon and that such degradation is, in most instances, the prime mechanism by which dalapon is inactivated or removed from the soil. Specific studies on the toxicity of dalapon to soil microorganisms, as well as studies on metabolism of dalapon by microorganisms, are summarized in Appendix A and have been discussed in Section 2.1. In general, degradation of dalapon by soil microorganisms is temperature sensitive and is inhibited at temperatures below 68 to 77°F (20 to 25°C). Dalapon degradation is also inhibited by low soil moisture, low (acidic) pH, and large amounts of organic material (Holstun and Loomis 1956 in Kenaga 1974).

Both dalapon and its sodium salt undergo chemical degradation by hydrolysis. However, since the hydrolytic half-lifes of both dalapon and its sodium salt appear to be in the order of several months at temperatures below 77°F (25°C), it is more likely that dalapon herbicides will be biologically degraded by soil microorganisms, which essentially degrade all of the applied material within one month under ambient environmental conditions (Kenaga 1974).

The potential for volatilization of dalapon from soil following forest applications is discussed by Ghassemi et al. (1981) in a review of studies on dalapon volatilization in laboratory and field environments. While the acid form of dalapon appears to be volatile, the sodium salt of dalapon does not volatilize to any great extent. Since the sodium salt of dalapon is widely used and, when applied to soils it does not convert to the volatile acidic form, it is unlikely that any significant amounts of dalapon will be lost from the soil by volatilization before it is degraded by soil microorganisms.

3.3 Water

Due to its high degree of solubility in water and its low adsorption onto soil particles, dalapon should not be readily removed from streams, ponds, and ditches by adsorption to sediments. Chemical degradation of dalapon by hydrolysis occurs only at a very slow rate (Section 3.2) and volatilization of dalapon results in a loss of only negligible amounts of the herbicide. Consequently, the

primary means for inactivation or loss of potential effectiveness of dalapon in aquatic systems are by microbial degradation and by dilution.

Studies by Alexander and Bachelder (1972 in Kenaga 1974) indicated that organisms in soils, in aqueous city sewage, and in industrial sludges are capable of degrading dalapon and Dowpon® M. Comparison of microbial degradation of dalapon and Dowpon® M with other rapidly biodegradable compounds such as sucrose, dextrose, glutamic acid, acetone, and ethanol indicate that dalapon and Dowpon® M are just as readily biodegraded. Pyruvic acid, a metabolite of dalapon, is an essential constituent of the reversible energy cycle in cells and in all likelihood, some pyruvic acid from dalapon degradation, rather than being completely oxidized to carbon dioxide, participates in the normal cellular energy cycle.

An important use for dalapon is in the control of ditchbank vegetation in irrigation systems. For this reason, the potential for dalapon contamination of water used for crop irrigation, and potentially for human and animal consumption, is of concern. The expected water concentration of dalapon applied at a rate of 1 lb (74% a.i.)/ditchbank acre is estimated to be 0.2 ppm (Folmar 1976, 1978). Frank et al. (1970 in Kenaga 1974) measured dalapon concentrations in canal water following ditchbank applications of 6.7 to 20 lb dalapon/acre (7.5 to 22.4 kg/ha) and found maximum concentrations of dalapon in the water in treated areas to range from 0.023 to 0.365 ppm 30 minutes after treatment. After several hours, only very low levels of dalapon (<10 ppb) were in the canal water flowing from the treated areas. The amount of dalapon that would be deposited by irrigation water with this concentration of dalapon would not be likely to adversely affect irrigated crops. Bruns and Dawson (1959 in Kenaga 1974) examined growth of crops irrigated with water containing dalapon sodium salt equivalent to 0.5 to 5.0 lb/acre (0.56 to 5.6 kg/ha) in three acre-inches of water. Corn was "injured" (nature and extent of damage not specified) at dosages of 2.5 and 5.0 lb/acre (2.8 and 5.6 kg/ha), but no damage was caused to rutabaga crops. Neither corn nor rutabagas were damaged when irrigated with the 0.5 lb/acre (0.56 kg/ha) dose of dalapon.

The above concentrations of dalapon in runoff water from ditchbank applications are well below toxic levels for aquatic organisms (see Appendices A and B). Although dalapon is highly mobile and does not adsorb readily to soil particles, it should be degraded by naturally occurring soil microorganisms before significant amounts reach waterbodies.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to dalapon during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved dalapon. Calculations of exposure to dalapon in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to dalapon are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Dalapon Application

Available application data for dalapon use by the USDA forest Service for 1982 are presented in Table 4-1. Actual exposure to dalapon differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of dalapon in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Dalapon is not readily adsorbed to soil. It has relatively high solubility in water and is readily leached from soil, but is also readily diluted and rapidly biodegraded. Dalapon has a very short soil persistence (less than one month). Consequently, the potential for incidental exposure of humans and wildlife to dalapon is low, existing only for a very short period immediately after its application.

4.2 Exposure

Estimates of potential exposure to dalapon are based on aerial as well as ground application. The maximum aerial application rate for dalapon (see Table 1-1) is 8.6 lb a.i./acre, while the compound is applied from the ground (ground foliar) at rates as high as

Table 4-1

USDA Forest Service Application Data for Dalapon (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Dalapon (2)	Aerial	7	14	5.3	1	5.8	1	Timber (2)
Dalapon (29)	Ground Foliar	8.5	247	7.1	13	8.6	14.8	Timber (24) Research (2) Wildlife (1) Recreation (1) Nursery (1)

[a]Grand Total = 261

Source: Gross 1983

30.0 lb a.i./acre. In both aerial application and ground foliar application, dalapon is applied as a soluble powder in a water spray. The calculations in Table 4-2 that are based on aerial application (e.g., spray/observer dose, wildlife dermal exposures, and water concentrations) assume an application rate of 8.6 lb a.i./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose, and wildlife oral exposures) assume an application rate of 30.0 lb a.i./acre to reflect a maximum reasonable dose/exposure situation. Other specific assumptions, based upon the toxicological and chemical characteristics of dalapon and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of dalapon to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for dalapon are based on doses on a per pound per acre application rate multiplied by 8.6 lb/acre for observers and 30.0 lb/acre for backpack sprayers. In the case of the aerial spray observer, dose estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see the General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. This absorption rate is based on the low solubility of dalapon salts in organic solvents and their relatively high water solubility, which would tend to reduce their ability to pass through the skin. At 8.6 lb a.i./acre aerial application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.344 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 30.0 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 1.20 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Dalapon

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg[b]		
Observer	up to 0.34 mg/kg		
Backpack Sprayer	1.20 mg/kg		
Environmental Exposure			
Fish	0.086 ppm (Immersion)[c]		
Rabbits	3.47 mg/kg	Negligible	31.50 mg/kg
Deer	0.87 mg/kg	Negligible	43.20 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.00052 mg/kg (2 days)
(Rabbits)	NA	NA	0.259 mg/kg (2 days)
(Deer)	NA	NA	0.347 mg/kg (2 days)
(Water)	NA	NA	0.0034 mg/kg (1 day)
(Berries)	NA	NA	0.0086 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0043 mg/kg (1 day)
Toxicity Summary	Undiluted solution causes necrosis and 10% solution is a skin irritant in rabbits	Not reported	Acute LD_{50}'s >2,000 mg/kg in rats, rabbits, mice, guinea pigs. Chronic feeding of 100 mg/kg in dogs and 50 mg/kg in rats increased kidney weight.
	Most studies, LC_{50} >50 ppm for fish		

[a]Assumes aerial application at 8.6 lb active ingredient per acre and ground foliar application at 30 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure.

[c]Fish exposure results from immersion in water containing dalapon.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by dalapon application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less (by orders of magnitude) than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 8.6 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 3.466 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.869 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus, a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of dalapon vapor would be negligible since the sodium salt of dalapon, the major

constituent of commercial formulations, volatilizes at a negligible rate. Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of dalapon in water, calculated as:

Water concentration: 0.086 ppm (0.01 ppm at 1.0 lb/acre aerial application rate)

This concentration of dalapon represents the estimated maximum concentration immediately after spraying. Although higher transient concentrations in water could occur in the event that high precipitation levels occur soon after dalapon application, the rapid degradation of dalapon and the high level of dilution that would occur with high precipitation levels make such higher dalapon concentrations unlikely.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of dalapon by workers on the spray unit would be accounted for by the estimtes of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed by ground broadcast equipment at 30.0 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 43.20 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 31.50 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of dalapon in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.086 ppm dalapon

reported actual dalapon concentration in fish tissue three times that of surrounding water)

Fish tissue concentration: 0.258 ppm (0.258 mg/kg)

Deer and rabbit tissue concentrations include dalapon accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied dalapon is absorbed at a rate of 10 percent as discussed in Section 4.2.1 with regard to spray observer absorption rates. It is also assumed that oral exposure of deer and rabbit to dalapon lasts only for 2 days due to the relatively short environmental persistence of dalapon. It was assumed that 20 percent of ingested dalapon would be absorbed by muscle tissue, based on metabolism data for mammals indicating rapid clearance of ingested dalapon and relatively lower residues in muscle tissue compared to tissues such as kidney and liver (refer to Appendix F). Deer tissue concentrations of dalapon are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.869 mg/kg (dermal exposure) x 0.10 = 0.0869 mg/kg.

Oral Exposure contribution assumes 20 percent assimilation of two-days feeding, or 43.20 mg/kg/day (oral exposure) x 0.20 x 2 days = 17.28 mg/kg.

Total Deer Tissue concentration = 0.0869 mg/kg + 17.28 mg/kg = 17.3669 mg/kg (17.367 ppm).

Rabbit tissue concentrations of dalapon are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 3.466 mg/kg (dermal exposure) x 0.10 = 0.3466 mg/kg

Oral Exposure contribution assumes 20 percent absorption of two-days feeding, or 31.5 mg/kg/day (oral exposure) x 0.2 x 2 days = 12.60 mg/kg.

Total Rabbit Tissue Concentration = 0.3466 mg/kg + 12.60 mg/kg = 12.9466 mg/kg (12.947 ppm).

These tissue concentration estimates are in the same range as muscle tissue concentrations from a variety of animals fed diets with significantly higher concentrations of dalapon than estimated here

for deer and rabbits (see Appendix F) and thus represent a conservative estimate of tissue residues. Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to dalapon from eating meat are derived:

Fish meat: 0.258 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0052 mg/kg

Deer meat: 17.367 mg/kg/ x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.347 mg/kg

Rabbit meat: 12.947 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.259 mg/kg

Water and plant consumption by man would result in the following oral exposures to dalapon based on assumptions stated in the General Introduction to the Herbicide Background Statements at an application rate of 8.6 lb a.i./acre (aerial):

Water:	0.0034 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries:	0.0086 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms:	0.0043 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of dalapon to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal effects in rabbits, rabbit and rodent acute oral LD_{50}'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for USDA Forest Service applications.

Small mammal studies have shown that dalapon applied to the eye and skin is irritating. Dalapon does not appear to be teratogenic. A single two-year feeding study employing a limited member of animals (20 to 24 per group) failed to reveal any carcinogenic potential of Dalapon sodium salt. No tests for mutagenicity have been reported. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive or carcinogenic effects to individuals from dalapon use, if proper care is taken during application.

Appendix A

Summary of Dalapon Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	References
Dalapon	Shrimp (*Crangon crangon*; Crustacea)	Surface absorption, static bioassay	24 hours	LC_{50} 100 ppm	--[b]	Portmann and Wilson 1971 in Folmar 1977
Dalapon	Cladoceran (*Daphnia magna*; Crustacea)	Surface absorption	48 hours	LC_{50} = 6 ppm	--	U.S. Department of the Interior 1968 in Pimentel 1971
Dalapon (Sodium salt)	Cladoceran (*Simocephalus serrulatus*; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 16.0 ppm	(11.4 to 22.4 ppm) 15°C temperature, 86.5% a.i., 1st instar	Johnson and Finley 1980
Dalapon (Sodium salt)	Copepod (*Cyclops sp.*; Crustacea)	Surface absorption	24 hours	100% mortality at 400 ppm (a.e.) 0% mortality at 200 ppm (a.e.)	Temperature = 80°F	Kenaga 1974
Dalapon (Sodium salt)	Entomostraca (*Eubranchipus sp.*; Crustacea)	Surface absorption	24 hours	100% mortality at 400 ppm (a.e.) 0% mortality at 200 ppm (a.e.)	Temperature = 80°F	Kenaga 1974
Dalapon (Sodium salt) (Nacan)	Harpacticoid (*Nitocra spinipes*; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} = 4,800 ppm	(3,970 to 5,810 ppm) 21 + 1°C. 73.5% active ingredient	Linden et al. 1979
Dalapon (Sodium salt) as Radapon	Brown shrimp (*Penaeus aztecus*; Crustacea)	Surface absorption, flow-through bioassay	48 hours	EC_{40} = 1.0 ppm (0.87 ppm a.i.)	40% of shrimp showed mortality or paralysis at 1.0 ppm.	Butler 1965
Dalapon (Technical)	Cladoceran (*Daphnia pulex*; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 11.0 ppm	(8.2 to 14.7 ppm) 15°C temperature 75.6% a.i., 1st instar.	Johnson and Finley 1980

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon	Bees (Apis mellifera; Insecta)	Ingestion	60 days	Reduced half-life of bee colony when bees fed 1,000 ppm dalapon by weight in 60% sucrose syrup.	Bees fed 10 and 100 ppm (by weight) dalapon lived as long as controls. Bees fed 1,000 ppm (by weight) had a half-life of 37.0 days, compared to a 51.8 day half-life for controls.	Morton et al. 1972
Dalapon (Sodium salt)	Stonefly (Pteronarcys californica; Insecta, nymphs)	Surface absorption, flow-through bioassay	96 hours	LC_{50} >100 ppm	No toxic effect.	Sanders and Cope 1968 in Folmar 1977
Dalapon (Sodium salt)	Stonefly (Pteronarcys sp., Insecta)	Surface absorption	24 hours	LC_{50} = 1.0 ppm	Nymphs.	Cope 1965 in Pimentel 1971
Dalapon (Sodium salt)	Dragonfly (Aeschna sp.; Insecta)	Surface absorption	48 hours	LC_{50} >1,600 ppm (a.e.)	No mortality observed.	Kenaga 1974
Dalapon	Mayfly (Ephemerella walkeri; Insecta)	Surface absorption	1 hour	Mayfly nymphs were attracted to water with 1.0 ppm dalapon but showed no response to or away from 0.1 and 10.0 ppm dalapon water.	—	Folmar 1978

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon	Heliodiaptomus viduus	Surface absorption	up to 792 hours	At 0.1 ppm, 50% mortality in 315 hours and 100% mortality in 648 hours. At 1.0 ppm, 50% mortality in 333 hours and 100% mortality in 348 hours. At 10.0 ppm, 50% mortality in 193 hours and 100% mortality in 768 hours. At 100.0 ppm, 50% mortality in 461 hours and 100% mortality in 792 hours.	Higher mortality at low doses may be due to injury to animals during collection or transport to lab. However, other studies support greater toxicity of dalapon at lower doses.	George et al. 1982
Dalapon (Sodium salt)	Honeybee (Apis mellifera; Insecta)	Surface absorption (wetted in solution)	"Momentarily"	No mortality observed 24 hours after immersion in 20,000 ppm solution.	—	Kenaga 1974
Dalapon (Technical, 75.6% a.i.)	Stonefly (Pteronarcys californica;) Insecta)	Surface absorption	96 hours	LC_{50} >1,000 ppm	First year class, 15°C temperature.	Johnson and Finley 1980
Dalapon	Cockle (Cardium edule; Mollusca)	Surface absorption, static bioassay	24 hours	LC_{50} >100 ppm	—	Portmann and Wilson 1971 in Folmar 1977
Dalapon (Sodium salt)	Snail (Planorbis sp.; Mollusca)	Surface absorption	—	50% mortality at 400 ppm (a.e.) 0% mortality at 200 ppm (a.e.)	—	Kenaga 1974
Dalapon (Sodium salt)	Snail (Physa sp.; Mollusca)	Surface absorption	—	50% mortality at 400 ppm (a.e.) 0% mortality at 200 ppm (a.e.)	—	Kenaga 1974

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon (Sodium salt) as Radapon®	Oyster (Crassostrea virginica; Mollusca)	Surface absorption, flow-through bioassay	96 hours	LC_{50} > 1.0 ppm (0.87 ppm a.i.)	No effect on shell growth, no toxic effect.	Butler 1965
Dalapon	Nematodes	Field exposure, surface absorption	—	94% reduction in nematode population	Dalapon applied at 5 lb/acre.	Courtney et al. 1962 in Pimentel 1971
Dalapon	Soil Organisms (Millipedes, Springtails, Mites, and Earthworms)	Field exposure, surface absorption	—	Increased populations of springtails and mites. No change in populations of earthworms.	Dalapon applied at 20 lb/acre.	Fox 1964 in Kenaga 1974
Dalapon	Soil bacterium (Rhizobium sp.)	—	—	Rhizobium found to be capable of using dalapon as its sole source of carbon and energy.	—	Berry et al. 1979
Dalapon	Soil bacterium (Rhizobium sp.)	Dalapon incorporated into agar culture medium.	—	Some strains of Rhizobium were sensitive to dalapon at 72.6 g/ℓ and other strains were not.	Rhizobium is symbiotic with legume plants and is important in their nitrogen fixation.	Faizah et al. 1980
Dalapon	Marine Unicelluar Alage: Chlorococcum sp.	Cultured in test-tubes with artifical sea water with 30 pp thousand salt.	10 days	EC_{50} = 50 ppm; EC_{100} = 100 ppm	Cultured at 20°C. Dalapon found to be 4 to 25 times more toxic on the basis of 10 days growth than Dowpon® M. A 100-fold difference in oxygen production inhibition occurred between dalapon and Dowpon® M with greater inhibition with dalapon.	Walsh 1972 in Kenaga 1974
	Phaeodactylum tricornutum			EC_{50} = 25 ppm; EC_{100} = 35 ppm;		
	Dunaliella tertiolecta			EC_{50} = 100 ppm; EC_{100} = 175 ppm;		
	Isochrysis galbana			EC_{50} = 20 ppm; EC_{100} = 35 ppm		

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon	Blue-green Algae: Aulosira sp., Tolypothrix sp., Anicystis sp., Anabaena sp. and Nostoc sp.	—	15 days	Algae were tolerant to dalapon at dosages equivalent to application rates of 179 lb/acre (200 kg/ha).	—	Venkataraman and Rajyalakshmi 1971 in Kenaga 1974
Dalapon	17 Strains of Algae (Chlorophyceae)	Herbicide incorporated into culture medium.	—	—	Two of 17 strains showed reduced growth with dalapon up to 10 ppm.	Cullimore 1975
Dalapon (Sodium salt)	Bacteria, Bacterial Spores, Algae, Actinomycetes, and Fungi	Herbicide incorporated into soil samples.	32 weeks incubation in the laboratory.	At concentrations of 2.6 and 26 ppm, dalapon had little effect on microorganisms. At 266 and 2,660 ppm, marked effects were observed. In one soil, a loamy sand, 2,660 ppm dalapon almost completely inhibited nitrification. All concentrations increased mineralization of nitrogen.	—	Greaves et al. 1981
Dalapon (Sodium salt) as Radapon®	Phytoplankton	Surface absorption flow-through bioassay	4 hours	No effect at 1.0 ppm (0.87 ppm a.i.)	No decrease in productivity (carbon fixation) observed.	Butler 1965

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon (80% wettable powder)	Soil Microbial Populations (Calcareous loam)	Herbicide incubated in soil at concentration equivalent to 5 ton/acre.	56 days	CO_2 evolution reduced by 21% to 24% compared to control.	--	Stojanovic et al. 1972
Dowpon® M	Marine Unicelluar Algae: Chlorococcum sp.	Cultured in test-tubes with artificial sea water with 30 ppm thousand salt.	10 days	EC_{50} = 500 ppm; EC_{100} = 1,000 ppm	Cultured at 20°C. Dalapon found to be 4 to 25 times more toxic on the basis of 10 days growth than Dowpon® M. A 100-fold difference in oxygen production inhibition occurred between dalapon and Dowpon® M with greater inhibition with dalapon.	Walsh 1972 in Kenaga 1974
	Phaeodactylum tricornutum			EC_{50} = 500 ppm; EC_{100} = 750 ppm		
	Dunaliella tertiolecta			EC_{50} = 400 ppm; EC_{100} = 800 ppm		
	Isochrysis galbana			EC_{50} = 500 ppm; EC_{100} = 750 ppm		

[a]95 percent confidence limits in parentheses.
[b]Data are not available or not applicable.

Appendix B

Summary of Dalapon Toxicity Data for Fish and Amphibians

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	References
Dalapon	Lake chub-sucker (fry)	Surface absorption, static bioassay	8 days	LC_{50} > 50.0 ppm	Temperature 22 to 25°C. All fry exposed 8 days, small fish (bluegill) exposed 12 days and eggs (green sunfish) exposed 3 days.	Hiltibran 1967
	Bluegill (small fish and fry)		8 to 12 days	LC_{50} > 50.0 ppm		
	Green sunfish (fry and egg)		3 to 8 days	LC_{50} > 50.0 ppm		
	Smallmouth bass (fry)		8 days	LC_{50} > 50.0 ppm		
Dalapon	Channel catfish (fingerlings)	Surface absorption, static bioassay	48 hours	Less than 10% mortality in 48 hours at 10 ppm dalapon.	—[b]	McCorkle et al. 1977
Dalapon	Goldfish	Surface absorption, static bioassay	96 hours	LC_{50} > 100 ppm	Preliminary tests.	Johnson and Finley 1980
	Rainbow trout		96 hours	LC_{50} > 100 ppm		
Dalapon	Harlequin fish	Surface absorption, static bioassay	48 hours	LC_{50} = 44 ppm	—	Alabaster 1969 in Folmar 1977
Dalapon	Flounder	Surface absorption, static bioassay	24 hours	LC_{50} > 100 ppm	—	Portmann and Wilson 1971 in Folmar 1977
Dalapon	Bluegill	Surface absorption, static bioassay	48 hours	LC_{50} = 115 ppm	Temperature 75°F.	Cope 1965 in Folmar 1977
Dalapon	Trout	Surface absorption, static bioassay	24 hours	LC_{50} = 340 ppm	—	Holden 1964 in Folmar 1977
Dalapon	(Common names not given) *Heteropneustes fossilis*	Surface absorption, static bioassay	8 days	Survived 8 days in concentrations ranging from 1 ppm to 2,500 ppm. Opercular beating rate inversely proportional to increasing dalapon concentration.	Preliminary data.	Rao and Dad 1979
	Sarotheroden mossambicus					
	Puntius ticto					

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon	Lake Emerald shiner	Surface absorption	72 hours	LC_{50} > 3,000 ppm	No toxic effects.	Springer 1957 in Pimentel 1971
Dalapon	Bluegill	Surface absorption, subchronic toxicity	7 days 15 to 21 days	LC_{50} >80 ppm LC_{50} >50 ppm	No mortality observed. No mortality observed.	Lawrence 1962a in Kenaga 1974; Lawrence 1962b in Kenaga 1974
Dalapon	Major carp	Surface absorption, static bioassay; subchronic	96 hours	100% mortality at 100 ppm (1.5 hours). 100% mortality at 50 ppm (24 hours). 10% mortality at 25 ppm. 0% mortality at 25 ppm.	Fingerlings.	Singh and Yadav 1978
Dalapon	Mosquito fish	Surface absorption, static bioassay	24 hours 48 hours 96 hours	Tl_{50} > 26,600 ppm TL_{50} > 23,100 ppm TL_{50} >19,100 ppm	21 to 22°C	Johnson 1978
Dalapon (85% a.i.)	Grass Carp	Surface absorption, static bioassay	24, 48, and 96 hours	LC_{50} >30,000 ppm	Temperature = 18 to 21°C; pH = 8.1; Hardness = 270 ppm. No mortality observed.	Tooby et al. 1980
Dalapon (Granular)	Bluegill	Surface absorption, static bioassay	24 hours 48 hours	LC_{50} >1,000 ppm LC_{50} >1,000 ppm	Fingerlings.	Hughes and Davis 1962 in Folmar 1977
Dalapon (Wettable powder)	Bluegill	Surface absorption, static bioassay	24 hours 48 hours	LC_{50} >1,000 ppm LC_{50} >1,000 ppm		
Dalapon (Technical 75.6% a.i.)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 105 ppm	Temperature = 24°C. Fish weight = 1.0 gram.	Johnson and Finley 1980
Dalapon (Sodium salt)	Longnose killfish	Surface absorption, flow-through bioassay	48 hours	LC_{50} > 1 ppm	Natural sea water. No toxic effect.	Butler 1965
Dalapon (Sodium salt)	Harlequin fish	Surface absorption, static bioassay	48 hours	LC_{50} = 240 ppm	Temperature 20°C.	Alabaster 1969 in Folmar 1977

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon (Sodium salt)	Goldfish	Surface absorption, static bioassay	24 hours	0% mortality at 100 ppm. 100% mortality at 500 ppm.	LC_{50} 100 ppm in static water bioassay tests with 12 species of fish.	Dow Chemical Company data cited in Weed Science Society of America 1979
Dalapon (Sodium salt, 86.5% a.i.)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} is 500 to 1,000 ppm	Preliminary data.	Johnson and Finley 1980
Dalapon (Sodium salt, Nacan®)	Bleak	Surface absorption, static bioassay	96 hours	LC_{50} = 5,500 ppm	(4,470 to 6,780 ppm) 10°C temperature, 73.5% a.i.	Linden et al. 1979
Dalapon (Sodium salt, powder, 74% a.i.)	Rainbow trout	Surface absorption, flow-through bioassay	96 hours	LC_{50} = 340 ppm	Fry. Tended to avoid water with 1.0 and 10.0 ppm dalapon but showed no significant response to or away from water with 0.1 ppm dalapon.	Folmar 1976
Dowpon®	Anura; Adelotus brevis	Surface absorption, static bioassay	24 hours 48 hours 96 hours	TL_{50} = 11,100 ppm TL_{50} = 5,200 ppm TL_{50} = 4,200 ppm	Tadpoles of A. brevis had reduced temperature tolerance compared to controls after 96 hours in 4,000 ppm dalapon.	Johnson 1976
Radapon® (Sodium salt)	Fathead minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 390 ppm (hard water) LC_{50} = 290 ppm (soft water).	Temperature 25°C, LC_{50} value for ppm formulation.	Surber and Pickering 1962 in Folmar 1977 and in Kenaga 1974
Radapon® (Sodium salt)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 440 ppm	Temperature 25°C, soft water, LC_{50} value for formulation.	Surber and Pickering 1962 in Folmar 1977

[a]95 percent confidence limits in parentheses.
[b]Data not available or not applicable.

Appendix C

Summary of Dalapon Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon	Mallards	Ingestion	—[a]	Reproduction depressed in animals fed at levels of less than 25% of those which produced mortality.	—	U.S. Department of the Interior 1962 in Pimentel 1971
Dalapon	Chicken (eggs)	Injection	—	Decreased rate of hatching compared to controls at doses giving a concentration in the egg of 300 ppm or more. No effect at 200 ppm.	No teratogenic effects observed. Acetone solution used as carrier.	Dunachie and Fletcher 1970
Dalapon	Pheasant	Ingestion	24 days (adults) to 169 days (young)	5,000 ppm in diet resulted in 24% mortality in young and 90% mortality in adults.	5,000 ppm in diet equivalent to 250 mg/kg/day for adults and 431 mg/kg/day for young.	U.S. Department of the Interior 1964 and Dewitt 1962 in Kenaga 1974
Dalapon	Bobwhite	Ingestion	100 days	At 5,000 ppm in diet, 40% of young and 95% of adults died.	5,000 ppm in feed equivalent to 610 mg/kg/day for adult birds.	U.S. Department of the Interior 1963 and Dewitt 1962 in Kenaga 1974
Dalapon	Mallards	Ingestion	100 days	At 5,000 ppm in diet, 24% of young died. At 2,500 ppm in diet, 85% of adults died. At 1,000 ppm in diet, 68% of young and 94% of adults died.	—	U.S. Department of the Interior 1963 and Dewitt 1962 in Kenaga 1974

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dalapon (Sodium salt)	Japanese quail, Ring-necked pheasant, Mallard	Ingestion (in feed)	5 days	LC_{50} > 5,000 ppm in diet.	No mortality observed to 5,000 ppm in diet. Birds were 10 to 12 days old at start of test. Fed 5 days toxic diet, observed after 3 days of nontoxic diet.	Hill et al. 1975
Dalapon (Sodium salt)	Chicken (mixed sexes, chicks)	Ingestion	--	LD_{50} = 5,660 mg/kg	--	Paynter et al. 1960 in Kenaga 1974
Dalapon (Sodium salt)	Pheasant (adults)	Ingestion	10 days	5,000 ppm in diet resulted in 10% mortality in birds.	5,000 ppm in diet equivalent to 250 mg/kg/day for adult birds.	U.S. Department of the Interior 1964 and Dewitt 1962 in Kenaga 1974
Dalapon (Sodium salt)	Mallards, Ring-necked pheasants and Bobwhite	Ingestion	--	Female bobwhites fed toxic lethal doses (1,000 to 5,000 ppm in diet) did not lay fewer eggs than controls. Treated mallards and pheasants did lay fewer eggs than controls. There was no effect on viability or on hatching success of eggs from treated females compared to controls for either of the three species.	--	U.S. Department of the Interior 1963 and Dewitt 1962 in Kenaga 1974
Dalapon (Sodium salt, 85% wettable powder)	Chickens	Ingestion (by capsule)	10 days	NOEL at 100 mg/kg body weight. Decreased weight gain at 250 and 500 mg/kg compared to controls.		Palmer and Radeleff 1969

[a]Data not available or not applicable.

Appendix D

Summary Dalapon Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Dalapon (Sodium salt)	Rat (male)	Single oral dose by intubation.	LD_{50} = 9,330 (8,460-10,300) mg/kg[a]	—[b]	Paynter et al. 1960
	Rat (female)		LD_{50} = 7,570 (6,880-8,350) mg/kg	—	
	Mouse (female)		LD_{50} > 4,600 mg/kg		
	Guinea pig (female)		LD_{50} = 3,860 (2,760-5,430) mg/kg	—	
	Rabbit (female)		LD_{50} = 3,860 (2,300-6,500) mg/kg	—	
Dowpon® Grass killer (85% dalapon sodium salt)	Rat (male and female)	Single oral dose by intubation.	LD_{50} = 5,660 mg/kg	—	Kenaga 1974
	Guinea pig (female)		LD_{50} = 4,930 mg/kg	—	
	Rabbit (female)		LD_{50} = 2,830 mg/kg	—	
Dowpon®M Grass killer (72.5% dalapon sodium salt and 12% dalapon magnesium salt)	Rat (male)	Single oral dose by intubation.	LD_{50} = 5,660 mg/kg	—	Kenaga 1974
	Guinea pig (female)		LD_{50} = 2,830 mg/kg	—	
	Rabbit (female)		LD_{50} = 2,140 mg/kg	—	
			Subchronic Oral Toxicity		
Dalapon (Sodium salt)	Cattle	Daily oral capsule, 500 mg/kg, for 10 days	None	—	Kenaga 1974
	Cattle	Daily oral drench, 250 mg/kg for 10 days; 500 mg/kg for 9 days	None at 250 mg/kg; throat irritation at 500 mg/kg	—	
	Sheep	Daily oral drench, 50 and 100 mg/kg for 10 days	None	—	

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Continued)		
Dalapon (Sodium salt)	Sheep	Daily oral capsule, 100 mg/kg for 10 days; daily oral capsule, 100 mg/kg for 481 days	Ill but survived, 6% weight loss. 10% weight loss after 86 treatments.	—	Kenaga 1974
	Sheep	Daily oral drench, 250 and 500 mg/kg for 10 days	None at 250 mg/kg. Ill after 7 treatments at 500 mg/kg. Survived with 6% weight loss.	—	
Dalapon (Sodium salt)	Calf (female)	Daily oral intubation of 1,000 mg/kg for 10 days	General lassitude, diarrhea, coat roughness, loss of appetite, slight loss of weight, slowed pulse rate, discharge from eyes, and mild cyanosis of mucous membranes. Recovered completely 4 days following last dose.	—	Paynter et al. 1960
	Calf (male)	Daily oral intubation of 1,000 mg/kg for 10 days	None except for slight kidney involvement, i.e., swelling of convoluted tubules and hypertrophy of the glandular cells	—	Paynter et al. 1960
	Sheep	Daily oral dose of 100 mg/kg for 35 days	None	—	Paynter et al. 1960
	Cattle	Daily oral doses of 100 mg/kg for 3 5 days	None	—	Paynter et al. 1960
	Dog	One male and one female mongrel dog were given dalapon orally, by capsule, 5 days a week during an 80-day period. Doses were 50 mg/kg for the first 2 weeks and then adjusted upward weekly until a maximum dose of 1,000 mg/kg/day was reached.	Except for vomiting, there were no gross signs of systemic toxicity. Hematological and biochemical studies as well as urinalyses revealed no adverse effects. On autopsy, gross inspection revealed no changes or alterations associated with treatment.	—	Paynter et al. 1960

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Concluded)		
Dalapon (Sodium salt)	Rat	Male and female animals maintained for 97 days on diets containing 0, 0.115, 0.0346, 0.115, 0.346 or 1.15% dalapon sodium	In male rats, there was no effect at the 0.115% level (equivalent to 115 mg/kg) or below. In female rats there were slight increases in average kidney weights at the 0.115 and 0.0346% dietary levels. At the higher concentrations of 0.346 and 1.15%, there was no evidence of adverse effects as judged by mortality, and food consumption, hematology, and microscopic examination of lungs, heart and spleen. Growth retardation, increases in average weight of liver and kidneys, and slight histopathological changes in the liver and kidneys were observed.	—	Paynter et al. 1960
Dalapon (Sodium salt?)	Calves	Grassy pasture treated with 30 lb/acre. Calves grazed on pasture until vegetation turned brown	None	—	Goldstein and Long 1960 in Kenega 1974
	Calves	Animals fed on oats treated with 2.5, 5, 10 and 20 lb/acre	None	—	Goldstein and Long 1960 in Kenega 1974
	Sheep	Animals fed on oats treated with 2.5, 5, 10 and 20 lb/acre	None	—	Goldstein and Long 1960 in Kenega 1974
	Calf	Animal fed on Sudan grass treated with 20 lb/acre	None	—	Goldstein and Long 1960 in Kenega 1974
Dalapon (Sodium salt)	Hogs, sheep and cattle	12,500 ppm in drinking water for 2 weeks	None	—	Goldstein and Long 1960 in Kenaga 1974

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Dermal Toxicity		
Dalapon (Sodium salt)	Rabbit	Undiluted dalapon sodium or solutions were applied to intact or abraded skin of the shaven abdomen as 10 continuous applications in 14 days.	The undiluted material produced moderate hyperemia and slight superficial necrosis of intact skin after 3 or 4 applications. Healing without scarring was complete 1 week following final appli-appliction. On abraded skin, prolonged contact resulted in necrosis. A 10% solution was only slightly irritating to intact or abraded skin, and a 1% solution caused no irritation to either abraded or intact skin. There was no evidence that dalapon sodium penetrated intact skin in acutely or subacutely toxic amounts.	—	Paynter et al. 1960
			Eye Irritation		
Dalapon (Sodium salt)	Rabbit	Dry powdered material or 10% and 1% solutions placed into the eye	With the dry material, the immediate response was slight irritation of conjunctival membranes. This was followed by severe conjunctivitis and corneal injury. Healing was complete after several days with no permanent damange. Washing the rabbits eye immediately with water greatly reduced the amount of irritation. A 10% solution caused only slight conjunctivitis. No reaction whatsoever was produced by a 1% solution.	—	Paynter et al. 1960

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity		
Dalapon (Sodium salt)	Dog	15, 50, or 100 mg/kg/day administered by capsule, 5 days a week, for 52 weeks	Adverse effects limited to increases in average kidney weights in animals receiving 100 mg/kg/day. Histopathological examination revealed no significant differences in tumors from control and treated animals.	Complete blood counts, hematocrits, sedimentation rates, blood urea nitrogen determinations, bromosulphalein liver function tests, and urinalyses performed initially and at end of 13, 26, and 52 weeks. Gross and microscopic examination of various tissues performed.	Paynter et al. 1960
Dalapon (Sodium salt)	Rat	Fed in diets containing 100, 300 and 1,000 ppm, equivalent to 5, 15, and 50 mg of dalapon sodium salt/ kg/day, for 2 years	Adverse effects limited to increases in average kidney weights in animals treated with 50 mg/kg/day. Microscopic examination of tissues revealed no adverse effects	Complete blood counts and hemogloben determinations performed initially, and at 13, 26 52 and 104 weeks. Gross and histological examination of various tissues performed on animals selected at 26, 52 and nervous at 104 weeks.	Paynter et al. 1960
			Carcinogenicity		
Dalapon (Sodium salt)	Rat	Fed in diets containing 100, 300, and 1,000 ppm, equivalent to 5 15 and 50 mg of dalapon sodium salt/ kg/day for 2 years	Histological examination of tissues from representative animals sacrified at 104 weeks revealed no differences from controls. Findings specifically related to tumor formation were not reported.		Paynter et al. 1960

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Reproductive and Teratogenic Effects		
Dalapon	Rat	Administered daily by gavage at doses of 250, 500, 1,000, 1,500, or 2,000 mg/kg/day to pregnant animals from day 6 through 15 of gestation.	No teratogenic effects were noted. Fetal resorption rate was increased and weight gains for pregnant dams were lower than controls at the 1,500 and 2,000 mg/kg dosage levels. No effects were seen at 1,000, 500, or 250 mg/kg/day.	—	Thompson et al. 1971 in Kenaga 1974
Dalapon	Rat	Administered daily by gavage at doses of 500, 1,000, or 1,500 mg/kg/day from day 6 through 15 of gestation	No major dose related skeletal or visceral abnormalities were observed. Reduced weights of pups in the 1,000 and 1,500 mg/kg/day dosage levels were observed.	—	Emerson et al. 1971 in Kenaga 1974
Dalapon (Sodium salt)	Rat	Fed a diet containing 0.03, 0.1, or 0.3% dalapon sodium salt (equivalent to about 30, 100, or 300 mg/kg/day) for 3 generations.	No adverse effect on fertility, gestation, viability, lactation, or normal growth and maturation.	—	Paynter et al. 1960

[a]95 percent confidence limits in parentheses.
[b]Data not available or not applicable.

Appendix E

Summary Mutagenicity Data for Dalapon

A search of the literature revealed no information regarding the mutagenic potential of dalapon.

Appendix F

Summary of Metabolism Studies with Dalapon

Formulation	Organism	Dose	Comments	References
Dalapon	Chickens (laying white rock hens)	25 and 50 ppm dalapon in diet for 30 to 60 days.	At 50 ppm dalapon in feed for 30 days, highest residues were in blood and kidney (22 ppm in each). Lower values of 4 to 12 ppm were found in feathers, meat, fat, gizzard, and liver. Average residues in eggs were 1.4 ppm. At 25 ppm, residues in feed were generally half those at 50 ppm and 30-day residues were not significantly different than 60-day. Egg residues reached a maximum level of less than 2 ppm within 2 weeks.	Getzendaner 1968 in Kenaga 1974
Dalapon	Steers	10 30, and 100 ppm in diet for 4 weeks.	At 100 ppm dalapon in feed, highest residues were in blood (5.9 ppm) and in kidney (2.5 ppm). Lower values (1.1 to 1.7 ppm) were found in muscle, fat and liver. In steers fed 100 ppm and then removed from dalapon diet prior to slaughter, the half-life of dalapon in tissues was 2 days.	Getzendaner 1969b in Kenaga 1974

Appendix F (Continued)

Formulation	Organism	Dose	Comments	References
Dalapon (Sodium salt)	Dogs	500 mg/kg body weight, single dose	65 to 70% excreted in urine in 2 days.	Leasure 1964 in Kenaga 1974
Dalapon (Sodium salt)	Dogs	60-day daily dietary doses of 50 and 500 mg/kg body weight	25 to 53% of total dalapon excreted in urine. Half-life in blood was approximately 12 hours.	Hoerger 1969 in Kenaga 1974
Dalapon (Sodium salt)	Dogs	500 to 3,500 ppm (15 to 100 mg/kg) in diet 5 days a week for one year	At 3,500 ppm dalapon in feed, highest residues were in kidney (69 ppm), liver (52 ppm) and muscle (43 ppm). Only 10.0 ppm were in fat.	Paynter et al. 1960 in Kenaga 1974
Dalapon (Sodium salt)	Humans	5 doses of 0.5 mg/kg body weight (198.3 mg total)	50% of total excreted in urine within 18-days.	Hoerger 1969 in Kenaga 1974
Dalapon (Sodium salt)	Cow	200 ppm dalapon in dry fed for 8 weeks	Cows produced milk with 0.7 to 1.0 ppm dalapon within 3 to 5 days. This level remained constant for 8 weeks. No dalapon was found in fat of milk.	Kutschinski 1961 in Kenaga 1974
Dalapon (Sodium salt)	Rats	100 to 1,000 ppm in diet for 2 years	At 1,000 ppm dalapon in feed, highest residues were in kidney (28 ppm), milk (19 ppm), and liver (11 ppm). Only 7.5 ppm were in muscle and 1.4 ppm in fat.	Paynter et al. 1960 in Kenaga 1974

Appendix F (Concluded)

Formulation	Organism	Dose	Comments	References
Dalapon (Sodium salt)	Pigs	100 to 200 ppm (4.7 to 8.6 mg/kg/day) in diet for 28 and 49 days	At 200 ppm dalapon in feed for 28 days, residues were: kidney, 10.4 ppm; liver, 6.8 ppm; lean meat, 5.8 ppm; and fat, 2.4 ppm. No increase in residues were observed when feeding was extended from 28 to 49 days.	Getzendaner 1969a in Kenaga 1974
Dalapon (Sodium salt)	Fish, snails, water fleas (Daphnia sp.) submerged plants, floating plants	In aquarium with 5 ppb, dalapon for 3 days. Second aquarium (control) contained both dalapon and dead organisms to differentiate physical adsorption from biological accumulation (absorption).	Concentration in water remained 5 to 6 ppb, fish tissue (dead and alive) contained up to 15 ppb, snails and Daphnia sp. contained less dalapon than surrounding water, submerged plants contained 20 to 50 ppb when dead and 780 ppb when alive (and still absorbing), and floating vegetation contained 40 to 50 ppb when dead and up to 180 ppb when alive.	Smith et al. 1972 in Kenaga 1974
Dalapon (Sodium salt, ^{36}Cl)	Cow	Not given	Only 2 labeled compounds, dalapon and chloride ion, were found in milk of cow fed labeled dalapon.	Redemann and Hamaker 1959 in Kenaga 1974

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Herbicide Background Statement: Dicamba

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Dicamba Summary

Major Trade Names: Banex®, Banvel®, Banvel D®, and Brush Buster®.

Chemical Name: 3,6-dichloro-o-anisic acid (or 2-methyl-3,6-dichlorobenzoic acid)

Major Forestry Application: Site preparation, range, and wildlife habitat management, noxious weed control, firebreak maintenance, general weed control, thinning, and rights of way. Dicamba controls annual and perennial broadleaf weeds and brush species resistant to phenoxy herbicides.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Dicamba is generally nontoxic to a wide variety of nontarget organisms. Studies with invertebrates and microorganisms show, in general, median lethal concentrations in excess of 100 ppm. It is only slightly toxic in fish and amphibians with LC_{50}'s in excess of 10 ppm. Acute oral toxicities in birds were equal to or greater than 673 mg/kg body weight.

In experimental studies with mammals, dicamba was a mild skin irritant and moderate skin sensitizer. Direct application of dicamba caused transient low grade eye irritation. The inhalation toxicity of dicamba was very low. Acute and subchronic ingestion of dicamba in laboratory animals resulted in slight toxicity. In isolated case reports, oral ingestion of dicamba by sheep caused death. Chronic consumption of dicamba in the diet by rats and dogs for two years elicited no adverse health effects, but chronic consumption by mice caused decreased body weight and increased liver weight. In rats, dicamba caused no reproductive or teratogenic effects. In rabbits, dicamba caused post-implantation losses, a decreased number of live fetuses, and decreased fetal weights. Dicamba is not considered to be mutagenic.

Environmental Fate

Dicamba is taken up by both roots and leaves of plants and translocated, generally to leafy tissue, or exuded by leaves and roots. Dicamba is readily metabolized in some plant species. It does not bioaccumulate to any appreciable extent in animals, from which it is rapidly excreted in the urine either as a metabolite or as parent dicamba.

Dicamba is moderately persistent in soil and water. It does not adsorb readily to soil particles and is highly mobile. It is primarily lost from soil and water by microbial decomposition.

Exposure and Hazard

There should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from dicamba at levels of application used by the USDA Forest Service if proper care is taken during application. Dicamba is mildly irritating to the eye and to the skin.

1.0 General Information

1.1 Normal Use Patterns

Dicamba (also known by the trade names* Banex®, Banvel®, Banvel D®, and Brush Buster®) is a selective herbicide for both preemergence and postemergence control of perennial and annual broadleaf weeds and brush. In addition to agricultural uses, such as on grain, corn, pasture, and range, dicamba is used for right-of-way maintenance, forest site preparation, and control of deciduous trees and shrubs as well as conifers (Ghassemi et al. 1981 and Weed Science Society of America 1983). Dicamba is absorbed rapidly by both roots and foliage, and is thus effectively applied as a ground or aerial spray and by basal application. It is also applied basally in the form of granules. Once absorbed, dicamba is readily translocated within the plant where it acts like a plant growth regulator. As such, it alters shoot and root development, resulting in leaf malformations, increased branching, and curvature of stems and leaf petioles. It also interferes with normal flowering mechanisms and results in destruction of plant cells (Frear 1975).

Dicamba is used selectively against a wide variety of broadleaf weeds and to control broadleaf weeds and brush species that are tolerant to other (phenoxy) herbicides. It is registered for use on crops such as small grains, corn, perennial seed grasses, and turf, as well as for noncrop applications. It is also useful on sorghum, pasture, sugarcane, and asparagus. On the other hand, legumes (beans and peas) are sensitive to dicamba, and conifers sprayed with dicamba are subject to top-kill and defoliation (U.S. Department of Agriculture 1978 and Weed Science Society of America 1983).

Statistics on the use of dicamba by the USDA Forest Service are presented in Table 1-1. Due to its high phytotoxicity to conifers, forest timber management applications are primarily for site preparation. Other forestry applications include range and wildlife habitat management, noxious weed control, nursery applications, and fire break maintenance (Gross 1983 and Ghassemi et al. 1981). Application rates range from 0.2 to 6.0 lb acid equivalent (a.e.) (formulated with 2,4-D) per acre for 44 USDA Forest Service projects in which dicamba was used in 1982 (Gross 1983). Application rates for dicamba as reported in Weed Science Society of America (1983) are as follows: 0.5 lb/acre (0.6 kg/ha) for preemergence; 0.06 to 0.25 lb/acre (0.07 to 0.3 kg/ha) for foliar spray on broadleaf annuals; and 0.5 to 10 lb/acre (0.6 to 11.2 kg/ha) for foliar spray on broadleaf perennials and brush.

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service Dicamba Use

Purpose	Active Ingredient Per/Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
1981					
Ground					
Conifer release[a]	0.2 - 1.8	1.8	485	Liquid	Stump spray Injection
Noxious weeds[b]	0.9 - 3.7	1.3	2,815	Liquid	Single plant Broadcast
Range improvement	1.0 - 2.0	1.7	105	Liquid	Broadcast
General weed control[c]	0.7	0.7	106	Liquid	Spot
Thinning[d]	0.2	0.2	41	Liquid	Injection
Right-of-way[e]	1.6 - 2.0	1.6	311	Liquid	Broadcast
Wildlife habitat improvement[f]	0.6	0.6	116	Liquid	Cut stump Injection
1982					
Aerial (1 project) Firebreak[g]	--[h]	1.4	198	Liquid	Aerial
Ground (28 projects) Range, Noxious Weeds, Nursery[g]	0.5 - 6.0	2.1	2,713	Liquid	Ground foliar
Timber, Range, Noxious Weeds	0.5 - 6.0	3.6	846	Pellet	Ground broadcast (Pellet)
Timber, Wildlife	0.3 - 5.0	1.7	832	Liquid	Cut surface (Injection)
Timber, Wildlife[g]	0.2 - 6.0	2.6	1,039	Liquid	Basal spray (Oil carrier)

[a]391 acres treated with dicamba + 2,4-D

[b]423 acres treated with dicamba + 2,4-D

[c]106 acres treated with dicamba + 2,4-D

[d]41 acres treated with dicamba + 2,4-D

[e]311 acres treated with dicamba + 2,4-D

[f]116 acres treated with dicamba + 2,4-D

[g]Treated with dicamba + 2,4-D

[h]Not available or not relevant.

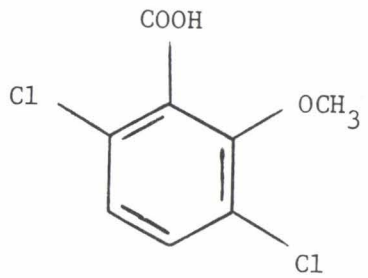
Source: Gross 1983

Dicamba may be applied by a variety of means, as ground or aerial spray, as granules, and as a basal application. Generally, water is used as the carrier and the mixture is applied at 2 to 4 gallons per acre. Additional surfactants are not required. Attapulgite, vermiculite, and liquid and dry fertilizers may also be used as carriers (Weed Science Society of America 1983).

Dicamba is manufactured and marketed in the United States by the Velsicol Chemical Corporation. It is available as a water soluble dimethylamine salt, as an oil-soluble acid formulation, and in granular form. It is also formulated with other herbicides--with 2,4-D as Banvel®, Weedmaster®, and Banvel K®; and with MCPA (a phenoxy herbicide) as MonDak® and Banvel M® (Meister Publishing Company 1981 and Weed Science Society of America 1983). Formulations of dicamba are described in Table 1-2.

1.2 Physical and Chemical Properties

The active ingredient of dicamba and its formulations is 3,6-dichloro-o-anisic acid (or 2-methoxy-3,6-dichlorobenzoic acid) or its salts. It has the following structure:



Dicamba has a molecular formula of $C_8H_6Cl_2O_3$ and a molecular weight of 221.0. At room temperature, it is an odorless, white, crystalline solid, although the technical grade material is a brown crystalline solid. Dicamba has a melting point of 237 to 241°F (114 to 116°C) and is relatively volatile, with a vapor pressure of 3.75×10^{-3} mm mercury at 212°F (100°C). The acid form of dicamba is relatively insoluble in water (0.45 grams per 100 ml water at 77°F [25°C]) but it is highly soluble in most organic solvents at 77°F (25°C) (92.2 grams per 100 ml ethanol, 5.2 grams per 100 ml heavy aromatic naphthanes, and 7.8 grams per 100 ml xylene). The alkali metal and amine salts of dicamba are highly soluble in water; the diethylamine salt of dicamba has a solubility greater than 72 grams per 100 ml water. In addition, dicamba is resistant to acid and to strong alkali and resists photolysis, oxidation, and hydrolysis under most environmental conditions. As a result of this resistance, most formulations of dicamba are extremely stable (Fear 1975, Melnikov 1971 and Weed Science Society of America 1983).

Table 1-2

Formulations of Dicamba

Formulation	Active Ingredient	Federally Registered Uses
Banvel® Herbicide (water soluble liquid)	4 lbs/gal dicamba (DMA salt)	Field corn, Small grains, Sorghum, Pastures and Rangeland, Noncropland, Grass grown for seed, Tree injection, Harvest aid-Sorghum, Cropland rotated to wheat
Banvel® 4S (water soluble liquid)	4 lbs/gal dicamba (DMA salt)	Lawns and Turf
Banvel® 5G (granules)	5% dicamba (free acid)	Pasture, Rangeland, Noncropland
Banvel® 4 O.S. (oil soluble liquid)	4 lbs/gal dicamba (free acid)	Industrial (tank mix with 2,4-D or 2,4,5-T only)
Banvel® 4 W.S. (water soluble liquid)	4 lbs/gal dicamba (DMA salt)	Industrial (alone or tank mix with 2,4-D or 2,4,5-T)
Banvel® 10G Granules	10% dicamba (free acid)	Brush
Banvel® 2% Granules	2% dicamba	Lawns and Turf
Banvel® CST (liquid)	1 lb/gal dicamba	Forest, Non-cropland, Rights-of-way
Banvel® XP (pellets)	10% dicamba (free acid	Pasture, Rangeland, Noncropland

[a]Formulations of dicamba with 2,4-D, 2,4,5-T, and MCPA are excluded from this list.

Source: Velsicol Chemical Company data cited in Ghassemi et al. 1981.

2.0 Toxicity of Dicamba

The toxiciological properties of dicamba, summarized in Table 2-1, show that this herbicide is generally nontoxic to a wide variety of nontarget organisms. There is no evidence for carcinogenic or mutagenic hazard from animal studies. There is evidence for reproductive effects in rabbits.

2.1 Invertebrates and Microorganisms

Studies of dicamba toxicity with invertebrates and microorganisms, summarized in Appendix A, indicate that dicamba generally has a low toxicity to these organisms. In general, median lethal concentrations of dicamba are in excess of 100 ppm, although LC_{50}'s of 10 ppm or less were reported for scuds (Gammarus sp., a Crustacean) in several studies.

For the most part, toxicity studies with invertebrates and microorganisms have been done in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily represent a true reflection of the potential effects of dicamba in a natural environment. For example, in the laboratory, aquatic organisms were generally kept up to 4 days or more in static conditions (in tanks or aquaria without a flow-through of fresh water). Microorganisms were grown in culture media in the laboratory and, in one study, microorganisms were cultured at dicamba concentrations equivalent to 5 tons per acre. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to dicamba or to other man-made chemicals is usually a transient phenomenon whose duration depends on the rate of streamflow or on the rate of decomposition of the chemical. Additionally, in natural systems, interactions with other man-made and naturally occurring chemicals, and changes in environmental factors, may affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions, in turn, potentially alter the tolerance levels of organisms to the herbicides.

Among the insect studies summarized in Appendix A, those with honeybees, representing insects of commercial importance, showed a variety of results generally indicating a low toxicity of dicamba to bees. An early ingestion study indicated an oral LD_{50} of 3.6 μg/bee (Edson and Sanderson 1965 in Pimentel 1971). On the other hand, Stevenson (1978) reported an oral $LD_{50} > 10$ μg/bee and Morton et al. (1972) found no increase in the rate of mortality in bees fed up to 1,000 ppm by weight of dicamba or of formulated Banvel® in sucrose for 60 days. In contact studies with bees, however, Stevenson (1978) found an $LD_{50} > 100$ μg/bee and Atkins (1975 in Ghassemi et al. 1981)

TABLE 2-1

Summary of Toxicological Properties of Dicamba

1. Acute Oral Toxicity

Organism (Formulation)	Adverse Effects
Rat (Dicamba, acid)	LD_{50} = 757 to 2,900 mg/kg
Rat (Dicamba, DMA salt)	LD_{50} = 1,028 mg/kg
Mouse (Dicamba, acid)	LD_{50} = 1,189 to >4,600 mg/kg
Guinea pig (Dicamba, DMA salt)	LD_{50} = 566 to 3,000 mg/kg
Rabbit (Dicamba, DMA salt)	LD_{50} = 566 to 2,000 mg/kg
Rat (Technical Banvel®)	LD_{50} = 1,707 to 2,900 mg/kg
Rat (Banvel® with DMA)	LD_{50} = 1,028 to 2,629 mg/kg
Sheep (Banvel D®)	Two doses of 500 mg/kg caused death, while 1 dose of 1,000 mg/kg caused no adverse effects.
Steer (Banvel D®)	Five doses of 250 mg/kg caused no adverse effects.
Fish (Banvel D® and Banvel® DMA salt)	24-hour LC_{50} = 20 ppm (Banvel D) to 96-hour LC_{50} >1,000 ppm (Banvel® dimethylamine salt)
Birds (various formulations)	LC_{50} = 673 mg/kg to 2,000 mg/kg

2. Acute Intraperitoneal Toxicity

Organism (Formulation)	Adverse Effects
Rat (Dicamba)	LD_{50} = 80 mg/kg

Table 2-1 (Continued)

3. Acute Dermal Toxicity

Organism (Formulation)	Adverse Effects
Rabbit (Dicamba)	Irritation reported with doses up to 2,500 mg/kg/day
Rabbit (Technical Banvel®)	LD_{50} > 2,000 mg/kg
Rabbit (Banvel® with DMA)	LD_{50} > 2,000 mg/kg
Rat (Dicamba, DMA salt)	Undiluted for 2 weeks caused very mild irritation; 1:40 dilution caused no irritation

4. Eye Irritation

Organism (Formulation)	Adverse Effects
Rabbit (Dicamba, DMA salt)	Mild irritation that disappears.
Rat (Dicamba, DMA salt)	Mild irritation that disappears.

5. Inhalation Toxicity

Organism (Formulation)	Adverse Effects
Rat (Banvel® and Banvel D®, (Technical)	LC_{50} > 9.6 to > 200 ppm for 4-hour dust exposure.

6. Subchronic Oral Toxicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Sheep (Banvel D®)	10 daily doses of 250 mg/kg	None
Rat (Dicamba)	650 to 23,500 ppm in diet for 3 weeks	No evidence of toxicity.
Rat (Dicamba)	5,000 to 15,000 ppm in diet for 4 weeks	Decreased weight gain. Posterior weakness at high doses.

Table 2-1 (Continued)

6. Subchronic Oral Toxicity (Concluded)

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Dicamba, acid)	800 ppm in diet for 3 months	None
Rat (Dicamba, (Technical)	5,000 to 10,000 ppm in diet for 13 weeks	10,000 ppm increased the number of cytoplasmic vacuoles in liver parenchyma.
Rat (Dicamba)	100 to 1,000 ppm in diet for 13 weeks	At 13 weeks liver and kidney pathology observed in 800 and 1,000 ppm groups.
Rat and Dog (2-methoxy-5-hydroxy-3,6-dichlorobenzoic acid)	100 and 250 ppm in diet for 90 days	None
Birds (various species, various formulations)	8-day dietary studies	$LC_{50} > 4,660$ ppm to $> 10,000$ ppm in feed

7. Teratology, Reproduction and Fertility

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Dicamba)	500 ppm in diet for 3 to 4 months	No teratogenic effects over three generations and no change in reproductive capability of parent or offspring.
Rabbit (Dicamba, Technical)	1 to 20 mg/kg/day on days 6 to 18 of gestation	High dose post-implantation losses and decreased number live fetuses. No teratogenicity below 10 mg/kg/day.

Table 2-1 (Concluded)

8. Carcinogenicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Beagle dog (Dicamba)	2 to 50 ppm in diet for 2 years	Tumor incidence and latency equal to untreated controls.
Mice (Dicamba)	100 to 10,000 ppm in diet for 14 to 19.5 months	No carcinogenic effect observed. At 10,000 ppm for 14 months increased mortality and liver weight and decreased body weight were observed.
Rat (Dicamba)	5 to 500 ppm in diet for 2 years	Tumor incidence and latency not different from untreated controls.

9. Mutagenicity

Organism (Formulation)	Adverse Effects
Bacillus subtilis	Recombination assay negative.
Salmonella typhimurium	
Strains TA98, TA100, TA1535, TA1537 TA1538	Histidine reverse mutation assay negative.
Saccharomyces cerevisiae D3	Mitotic recombination assay negative.
Escherichia coli WP2	Tryptophan mutation assay negative.
WI-38 human fibroblasts	Unscheduled DNA synthesis assay negative.
Bacteriophage	Mutant induction and reversion assay negative.
E. coli (W3110 and P3478)	Relative toxicity assays positive, indicating damage to DNA.
B. subtilis (H17 and M45)	

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendices.

reported a 2.6 percent mortality at 91 μg/bee. Such doses are far in excess of those that would result from normal application rates, since application at 1 lb/acre would result in a contact dose equivalent to approximately 1.25 μg/bee (Ghassemi et al. 1981). In a feeding study with another insect, the German cockroach, dicamba at 1,000 ppm in food had no observable toxic effect nor did it affect reproduction through two generations (Riviere 1976).

Several studies have been conducted with both marine and freshwater crustaceans and indicate that dicamba is only slightly toxic to these organisms. With the exceptions of one study reporting a 48-hour EC_{50} of 11 ppm dicamba for water fleas (Daphnia) (Sanders and Cope 1966 in Hurlbert 1975) and one study reporting a 96-hour LC_{50} of 56 ppm dicamba for shrimp (Paleomonetes) (Johnson and Finley 1980), LC_{50}'s, EC_{50}'s and TL_{50}'s for crustacea were all determined to be greater than 100 ppm.

Limited studies of the effect of dicamba on soil organisms indicate some reduction in growth at 50 ppm dicamba in medium for bacteria, actinomycetes, and fungi but not for yeasts (Cooper et al. 1978). Two of 17 strains of algae showed reduced growth when dicamba was incorporated into the culture medium at 10 ppm (Cullimore 1975). When dicamba was incorporated into soil cultures at a rate equivalent to 5 tons per acre, carbon dioxide production (a measure of microbial metabolism) was reduced by as much as 14 percent compared to controls during a 56-day period (Stojanovic et al. 1972).

2.2 Fish and Amphibians

The toxic effects of dicamba and several of its formulations on fish and amphibians have been reported in studies summarized in Appendix B. In all cases, LC_{50}'s were in excess of 10 ppm. Clark et al. (1970) suggest that pesticides with median lethal concentrations of 10 ppm or more for fish be considered "slightly toxic." Reported toxicities range from a 24-hour LC_{50} of 20 ppm in bluegills for Banvel D® adsorbed onto vermiculite (Hughes and Davis 1962 in Lorz et al. 1979) to a 96-hour $LC_{50} > 1{,}000$ ppm in bluegill for Banvel® dimethylamine salt (Velsicol Chemical Corporation data cited in Ghassemi et al. 1981).

In 1 to 2-week old frog tadpoles, dicamba toxicities ranged from a 96-hour LC_{50} of 106 ppm to a 24-hour LC_{50} of 220 ppm (Johnson 1976).

2.3 Birds

Dietary studies and egg injection bioassays with dicamba indicate a low toxicity to birds (Appendix C). Acute oral toxicities

of dicamba in birds ranged from an LD_{50} of 673 mg/kg in female pheasants (Edson and Sanderson 1965 in Pimentel 1971) to an LC_{50} of 2,000 mg/kg in mallards. In 8-day subchronic feeding studies with birds, dicamba toxicities were reported as LC_{50}'s 10,000 ppm for all species and formulations studied with the exception of a reported $LC_{50} > 4,600$ ppm for Banvel® 310 in mallards (Velsicol Chemical Corporation data and U.S. Environmental Protection Agency registration data cited in Ghassemi et al. 1981).

Dunachie and Fletcher (1970) reported a decreased rate of hatching compared to controls in chicken eggs injected with dicamba at doses of 400 ppm in the egg. No effect was observed at 300 ppm, although an anomalous low hatch due to early mortality was observed at concentrations of 200 ppm dicamba in the eggs. No teratogenic effects (e.g., feather blanching) were observed in this study.

2.4 Mammals

The potential health hazards from acute and chronic exposure to dicamba have been evaluated primarily in laboratory animals such as mice, rats, rabbits and guinea pigs. A few studies on dogs and observations of effects in sheep and cattle have also been reported. The results of the toxicity tests, summarized in Table 2-1, Appendix D, and Appendix E, are discussed below.

2.4.1 Acute Toxicity

Acute toxicity data have been reported for oral, intraperitoneal, dermal, ocular, and inhalation studies.

2.4.1.1 Oral. Based on the acute toxicity tests, dicamba is classified as slightly toxic (U.S. Department of Energy 1983). For the acid form of dicamba, the oral LD_{50}'s are reported to be 2,900 $\pm$ 800 mg/kg in rats and greater than 4,600 mg/kg in mice (U.S. Department of Energy 1982). For the dimethylamine salt of dicamba, the oral LD_{50} in rats is 1,028 to 2,629 mg/kg body weight (U.S. Department of Energy 1982 and Ghassemi et al. 1981). Dimethylamine (DMA) salt forms of dicamba give LD_{50}'s in the ranges of 566 to 3,000 mg/kg for guinea pigs and 566 to 2,000 mg/kg for rabbits (Appendix D).

2.4.1.2 Intraperitoneal. An intraperitoneal LD_{50} of 80 mg/kg in male rats has been reported for dicamba (Edson and Sanderson 1965).

2.4.1.3 Dermal. Applications of dicamba to the skin of rabbits and rats induce mild skin irritation. Dicamba causes skin irritation in rabbits administered doses up to 2,500 mg/kg/day with extended contact. No other signs of toxicity have been observed, although in one dermal study in rabbits, 7 of 32 rabbits that were tested died,

apparently due to nontreatment-related causes (U.S. Department of Energy 1983). In dose-response studies, rabbits were treated with dicamba 3 times weekly for 3 weeks, then 2 followup treatments were applied in 14 days and in 16 days. Moderate dermal sensitization resulted (U.S. Department of Energy 1983). In dose-response studies, daily administration for 2 weeks of the undiluted DMA salt of dicamba to the skin of rabbits and rats caused very mild irritation, while dilutions of 1:40 in water caused no irritation over a 30 day observation period (Velsicol 1971 in Weed Science Society of America 1983). Systemic toxicity from percutaneous absorption was not evident.

2.4.1.4 Eye Irritation. Applications to rats and rabbits of a 0.1 ml aqueous solution of the DMA salt of dicamba caused no injury to the cornea or iris, although a low grade irritation which disappeared rapidly was induced (Velsicol 1971 in U.S. Department of Agriculture 1981). Neither eye irritation nor injury have been reported to follow direct application of 0.2 to 2.0 percent aqueous solutions either as single doses or as repeated doses over a 1 week period (U.S. Department of Agriculture 1981).

2.4.1.5 Inhalation. In one inhalation study, the LC_{50} (4-hour dust exposure) has been reported to be greater than 9.6 ppm, species not reported (U.S. Department of Energy 1983) for Banvel®, technical grade. In another study, the LC_{50} has been reported to be greater than 200 ppm in rats (U.S. Department of Energy 1982 and Ghassemi et al. 1981) for Banvel® technical grade. This apparent discrepancy may be due to species differences. In another report (Velsicol Chemical Corporation 1979 in Ghassemi et al. 1981), the inhalation LC_{50}'s in rats for 4-hour exposure were greater than 200 ppm for both technical Banvel® DMA and Banvel® 310 (4 lb/gal. Dicamba as the DMA salt). Based on these data, inhalation toxicity is a slight health hazard since the toxicity category is III or greater, warranting handling with caution (as discussed in the General Introduction to these background statements).

2.4.2 Subchronic Toxicity

Exposure of rats or sheep to less than 500 ppm dicamba for periods up to 13 weeks causes no toxic effects. By contrast, in rats, dietary administration of very high doses of dicamba for intervals of 4 to 13 weeks induce mild toxicity. In a 3-week study, dietary concentrations of dicamba fed to one male and one female rat per dose level induced no toxic effects (U.S. Department of Energy 1982). However, with increases in group sizes to 5 rats of each sex per dose level (5,000, 7,500, 10,000, 12,500, and 15,000 ppm), weight gain decreased to 40 percent of controls, and 7 of 10 rats developed posterior weakness in the 15,000 ppm group. In a third study,

a lower dose of 800 ppm dicamba fed to rats for a longer period (3 months) elicited no adverse effects (Weed Science Society of America 1983). In two 13 week dietary studies, high doses of dicamba caused liver toxicity. In one study, the number of cytoplasmic vacuoles was reduced in liver parenchyma of rats fed 10,000 ppm dicamba (U.S. Department of Energy 1983). In the second study, feeding of 100 to 1,000 ppm dicamba yielded no deaths, normal food consumption, and normal growth rates throughout (U.S. Department of Agriculture 1981). Furthermore, in this study, while there were no adverse effects after 7 weeks of feeding, abnormal pathology in kidney and liver was observed after 13 weeks in the 800 and 1,000 ppm dose groups (U.S. Department of Agriculture 1981).

A principal metabolite of dicamba is 2-methoxy-5-hydroxy-3,6-dichlorobenzoic acid. Rats and dogs, fed 100 and 250 ppm of this metabolite for 90 days, developed no chemical, behavioral, or other evidence of toxicity (U.S. Department of Energy 1982).

Effects of dicamba on castrated male sheep (wether), have been reported (Palmer and Radeleff 1964). Ten daily doses of 250 mg/kg caused no effect in one wether. Two doses of 500 mg/kg killed another, while a single dose of 1,000 mg/kg did not kill a third wether. Also, in the report of Radeleff (1964), a yearling steer was not affected by five doses of 250 mg/kg.

2.4.3 Chronic Toxicity

The chronic toxicity of dicamba has been evaluated in rats, dogs, and mice which received dicamba in the diet for periods up to 2 years (Table 2-1) with the highest dose inducing increased mortality and organ weight changes in mice. Continuous feeding of rats with 5, 50, 100, 250, and 500 ppm dicamba for 2 years and of beagle dogs with 5, 25, and 50 ppm dicamba for 2 years elicited no adverse effects. Survival, body weight, food consumption, organ weight, hematology, and histology of viscera were normal (Weed Science Society of America 1983). In another chronic study, mice were fed dicamba at concentrations of 100, 1,000, and 10,000 ppm. At 10,000 ppm, decreased body weight and increased liver weight were observed. All high dose mice were sacrificed after 14 months of feeding. The medium and low dose groups were sacrificed 19.5 months after continuous dicamba feeding. Enlargement of liver cells was the only dose-dependent effect noted and whether this effect was reversible was not determined (U.S. Department of Energy 1983).

2.4.4 Reproductive and Teratogenic Effects

Reproductive and teratogenic effects of dicamba have not been observed in several rat studies; teratogenic effects have been

observed in rabbits at high doses. Rats fed 500 ppm dicamba for 3 to 4 months showed no change in reproductive capacity in either parents or offspring and produced no evidence of teratology over a three generation study (Velsicol 1971 in U.S. Department of Agriculture 1981). In this study, 2 males and 4 females fed 500 ppm dicamba were mated. In addition, 2 treated males and 4 treated females were mated with untreated rats. No reproductive effects were observed, and no changes in fertility, gestation, viability, or lactation were observed over three generations. In rabbits, however, a differential response to technical dicamba levels of 1, 3, 10, and 20 mg/kg/day given on days 6-18 of gestation has been reported. At 10 and 20 mg/kg/day, post-implantation losses and a decreased number of live fetuses were observed (U.S. Department of Energy 1983). With 3 mg/kg/day, there were no effects. A nontreatment-related decrease in post-implantation loss was observed in the low dose, 1 mg/kg/day, group. Decreases in fetal weight and post-implantation losses occurred at 10 mg/kg/day.

2.4.5 Carcinogenicity

Chronic feeding studies discussed above with dicamba have revealed no carcinogenicity in rats, beagle dogs, and mice. Dicamba was administered for 2 years in the diet of beagle dogs at 5 to 50 ppm levels and in the diet of rats at 5 to 500 ppm levels. The tumor incidence and time until appearance of the first tumor were the same in untreated controls and in groups of rats and dogs fed dicamba for 2 years (Weed Science Society of America 1979). Mice were administered 10,000 ppm dicamba in the diet for 14 months, and 100 or 1,000 ppm dicamba in the diet for 19.5 months. There was no evidence for dicamba-induced carcinogenicity in mice sacrificed either at 14 or 19.5 months (U.S. Department of Energy 1983).

2.5 Mutagenicity

Dicamba has been tested for mutagenicity and for its effect on unscheduled DNA synthesis (Appendix E). The microbial test systems and results have included the following: (1) the histidine reverse mutation assay in Salmonella typhimurium strains TA1535, TA 1537, TA1538, TA98 and TA100 was negative (Poole et al. 1977; Eisenbeis et al. 1981 and Anderson et al. 1972); (2) the tryptophan mutation assay in Escherichia coli WP2 was negative (Poole et al. 1977; (3) the mitotic recombination assay in Saccharomyces cerevisiae D3 was negative (Poole et al. 1977) and (4) the induction of rII mutants of T_4 bacteriophage and reversion of bacteriophage AP72 in E. coli were negative (Andersen et al. 1972). Unscheduled DNA synthesis was assayed in human fibroblast line WI-38 and was negative for dicamba (Poole et al. 1977). Assays 1-3 above and the unscheduled DNA synthesis assay used an activation system. This was prepared from

livers of Aroclor 1254 induced rats. Dicamba was positive in relative toxicity assays in E. coli (strains W3110 and P3478) and B. subtilis (strains H17 and M45) (Poole et al. 1977). For each of these bacterial species, the effect of dicamba on DNA repair proficient and DNA repair deficient strains have been compared. Dicamba has been negative in all of the above in vitro test systems except those measuring relative toxicity. On the basis of these results, dicamba is not considered to be mutagenic.

3.0 Environmental Fate

Dicamba is selectively toxic to plants including annual broadleaf and grassy weeds. It is moderately persistent in soils (3 to 12 months) giving full season control (Ashton 1982), is moderately persistent in water, and does not bioaccumulate in animals. Dicamba is absorbed by both plant roots and foliage and, once absorbed, is readily translocated, generally to leafy tissue. Once it is taken up by the plant, dicamba is metabolized at a rate that is dependent upon the plant species (Frear 1975). It does not bioaccumulate in animals.

3.1 Bioaccumulation and Metabolism

Bioaccumulation, metabolism, and toxicity of dicamba in plants have been reviewed by Frear (1975) and by Ghassemi et al. (1981). There are few studies on the bioaccumulation and metabolism of dicamba in animals. These studies are also summarized in Frear (1975) and Ghassemi et al. (1981).

3.1.1 Plants

Uptake of dicamba in plants is by both roots and foliage. Once in the plant, dicamba is readily translocated as unchanged dicamba, as the hydroxy-dicamba metabolite, or as a conjugate of dicamba with other organic metabolites of the plant (Frear 1975). The actual rate of absorption of herbicide, and the pattern of its translocation and final distribution within the plant, is highly species-specific.

In addition to being metabolically degraded by plants, dicamba can be exuded from roots and lost through the leaf surface. In a study of dicamba persistence in range forage grasses, Morton et al. (1967 in Ghassemi et al. 1981) found dicamba applied at 1 lb/acre to have a half life of 2 weeks in the grasses and 2.6 weeks in litter tissue (Brady 1975).

Some plant species are able to metabolize dicamba readily and rapidly. Frear (1975) has reviewed the mechanisms of metabolic degradation of dicamba in plants. Dicamba undergoes hydrolysis and/or loss of the methyl group with subsequent conjugation to a sugar or to other plant molecules, or direct conjugation of unchanged dicamba. Chang and Vanden Born (1971) examined the uptake, translocation, and metabolism of ^{14}C-labeled dicamba in four species of plants. They found the hydroxy-dicamba derivative to be the major metabolite in all 4 species, with an unidentified minor metabolite in 2 species resulting from demethoxylation.

3.1.2 Animals

Dicamba does not bioaccumulate to an appreciable extent in animals. Most of the residues excreted by animals in the studies of metabolism are in the form of the parent dicamba, although some is excreted as a conjugate with glucuronic acid and some is excreted as 3,6-dichloro-2-hydroxybenzoic acid. The limited studies of bioaccumulation and metabolism of dicamba in animals are summarized in Appendix F.

Studies with cattle (Oehler and Ivie 1980 and St. John and Lisk 1969 in Hayes 1982) and with rats (Tye and Engel 1967 in Paulson 1975 and in Hayes 1982) indicate that as much as 90 percent of dicamba was eliminated in the urine when administered by intubation, in feed, or by injection. This elimination in the urine occurred rapidly and, in one study (Oehler and Ivie 1980), was measured within 6 hours of final dosing. A short-term study in lactating dairy cattle has been reported (Velsicol 1971 in U.S. Department of Agriculture 1981). The cattle were provided 10, 25, 50, 80, and 400 ppm dicamba in the diet for 9 days. No residue was observed in the milk at 10, 25 and 50 ppm concentrations. Residues not exceeding 0.15 ppm were detected in the milk of lactating cattle after 9 days for 80 and 400 ppm dicamba levels.

Studies of tissue accumulations of dicamba in animals dosed by various routes indicate that dicamba does not bioaccumulate. When dicamba was fed to a cow at 2.2 mg/kg body weight (total of 4.95 grams over 5.5 days) at a rate equivalent to 60 ppm in feed, maximum tissue levels of 2.59 ppm were found in the kidney, while 0.02 to 0.03 ppm dicamba were found in muscle (Oehler and Ivie 1980). In a miniature ecosystem established in an aquarium, application of dicamba equivalent to a rate of 1 lb/acre resulted in dicamba levels of 0.02 ppm in fish tissue (Sanborn 1974 and Yu et al. 1975).

3.2 Soil

Dicamba has a moderate (3 to 12 months) persistence in soil compared with other herbicides, thus allowing full-season herbicidal control (Ashton 1982). Dicamba does not adsorb readily to soil particles and colloids, and this factor, coupled with the relatively high solubility of dicamba salts in water, results in a high degree of mobility in most soils. The major route for loss of dicamba in soil, however, appears to be through microbial degradation, although confirmatory direct evidence is lacking (Frear 1975 and Ghassemi et al. 1981).

A number of studies with dicamba have generally shown it to be moderately persistent in soil. Studies reviewed by Frear (1975) indicate that runoff losses and photodegradation are not major mechanisms for removal of dicamba from soil. Studies on volatilization of dicamba have given conflicting results indicating that dicamba may volatilize from soil surfaces but that further study is required to determine the extent of such loss.

In studies of dicamba persistence in soil, Stewart and Gaul (1977) applied dicamba to silt loam soil plots in late summer at rates equivalent to 1, 2, and 4 lb/acre and found residues as high as 0.07 ppm in the top 10 cm and 0.03 ppm in the 10 to 20 cm horizons 265 days later. After more than one year (385 days), only traces of dicamba were identified in the soil. Altam and Stritzke (1973) examined the half-life of dicamba incorporated in three forest soils from Oklahoma and found a rapid disappearance of dicamba for the first 20 days followed by a less rapid disappearance. They calculated the half-life of dicamba to range from 17 to 32 days in these soils. In prairie soils from Canada, Smith (1973 and 1977) failed to find dicamba residues in May following October application at 1 lb and 2 lb/acre, nor were they recovered in October following similar application in May. Lange et al. (1968) found that when dicamba was applied at 4 lb/acre in various California soils, minor residual effects (damage to broadleaf crops) occurred for more than a year after application. At application rates of up to 16 lb/acre, however, residual effects after one year were still only minor. Rahman et al. (1981) studied the residual activity of dicamba in various soils in New Zealand following application at rates up to 0.7 lb/acre (0.8 kg/ha) and found that residues were low enough for the establishment of clover within 6 to 7 weeks after application. They also determined that soil temperature and texture, as well as rainfall, all affected dicamba persistence, with more rapid loss of activity with higher temperature and in heavier soils (silt loam and clay loam). Hahn et al. (1969) reported results with dicamba similar to those of Rahman et al. (1981), finding that dicamba dissipation was greater in a silty clay loam than in a sandy loam and was faster at higher temperatures than at low temperatures.

In a comparative study of pesticide mobility in a silty clay loam, Helling (1971) found dicamba to be relatively mobile compared to 40 pesticides tested. He also found that pH had a strong influence on dicamba mobility, and that at pH less than 4.2, dicamba was not readily leached from soils. In the pH range of 5.0 to 6.7, dicamba mobility increased with increasing pH, but at pH levels above 6.7, there was no increase in dicamba mobility. Despite this high measured mobility of dicamba, Frank and Sirons (1980) found dicamba residues in only one of 949 water samples (0.1 percent) from 11 agricultural watersheds in which a total of 63 kg of dicamba had been

applied. In this sample, residues of 0.7 ppb dicamba were found following application of a total of 5 kg of dicamba for right-of-way maintenance. Application rate was not specified in this study.

Microbial degradation of dicamba as a function of soil pH was reported by Smith and Cullimore (1975). Radiolabeled dicamba (^{14}C-dicamba) was incorporated in laboratory soil samples (Canadian prairie soils) at a concentration of 2 ppm (equivalent to an application rate of 1.1 kg/ha or 1 lb/acre) and, following 4 to 19 days of incubation, dicamba was extracted from the soil samples and measured by determining the level of residual radioactivity. No dicamba loss was found in the soils at temperatures of -5°C, but from 5 $\pm$ 1°C to 35 $\pm$ 1°C, there was a direct relationship between temperature and degradation of dicamba. Since earlier studies by Smith (1973 and 1974) showed no dicamba degradation in these same prairie soils after steam sterilization, Smith and Cullimore (1975) attribute their observed degradation to microbial activity. Preliminary studies reported by Smith and Cullimore (1975) indicated large populations of various microbial organisms potentially capable of degrading dicamba. In general, however, the strongest support for the importance of microbial breakdown of dicamba is from indirect evidence; those environmental soil conditions in which dicamba persistence is reduced are also those environmental conditions most favorable to bacterial growth, while dicamba persistence is increased in conditions unfavorable to bacterial growth. Conditions favorable to bacterial growth include high organic matter, sufficient moisture, warm temperatures, and neutral pH (Frear 1975).

Chemical degradation and photodecomposition do not appear to be major routes by which dicamba is lost from soil. Smith (1974) found only negligible degradation of dicamba in steam-sterilized soils, although, in nonsterilized soil, dicamba was degraded relatively rapidly (over 50 percent of the dicamba degraded within 4 weeks). Hahn et al. (1969) found only slight loss in phytotoxicity of dicamba to cucumbers in soils exposed for 16 days to sunlight compared to controls. They concluded that this loss in toxicity could be attributable to biological degradation rather than to photodecomposition of dicamba, although the reason for this conclusion was not given.

Although laboratory studies indicate that dicamba is relatively volatile, there is conflicting evidence regarding the actual volatilization of dicamba from soils (Frear 1975). On the one hand, studies of soil-incorporated dicamba indicate little loss of dicamba over an 8-week period, while on the other hand, as much as 50 percent loss occurred on planchets over an 11-week period and vapors from soil-incorporated dicamba were toxic to beans. Frear (1975) concluded

from the above evidence that dicamba may volatilize from the soil surface, but that further work was necessary to define the extent and significance of such losses.

3.3 Water

The persistence and fate of dicamba in aquatic systems have been reviewed by Ghassemi et al. (1981). Because dicamba salts are highly water-soluble and rapidly enter the soil, it is unlikely that sufficient amounts of residues will remain readily available for transport, via precipitation runoff, into nearby waterbodies. Frank and Sirons (1980) found dicamba residues (0.7 ppb) in only one of 949 stream samples following dicamba application to watershed soils. Norris and Montgomery (1975) sampled a stream following treatment of 165.5 acres (67 ha) of a total 602.7 acre (244 ha) forest watershed in the Pacific Northwest treated with dicamba sprayed aerially at a rate of 1 lb/acre (1.12 kg/ha). Samples taken where the stream exited the watershed contained dicamba residues within 2 hours after start of spraying. These residues rose to a maximum of 37 ppb at 5.2 hours, and then dropped to background levels (less than 1 ppb) 37.5 hours after the start of spray. The authors attributed these residues to drift and to direct application of dicamba to water surfaces.

In a study of the dissipation of dicamba following application to pond surfaces, Scifres et al. (1973) found that dicamba applied at a rate equivalent to approximately 4 lb/acre (4.3 to 4.5 kg/ha) surface area (resulting in 11 ppm dicamba concentration) was completely dissipated after about 40 days. These same authors found, in laboratory studies, that dissipation was more rapid in water in nonsterile, lighted conditions than in sterile, dark conditions and that pond sediment appeared to contain microbiological organisms that were capable of decomposing dicamba.

Dicamba is relatively stable in water when microbiological degradation is prevented. Chau and Thompson (1978) reported on the stability of dicamba in distilled water and in natural lake water that had been acidified to kill microorganisms. There was no detectable degradation of dicamba in either distilled water over a 40-day period or in the natural lake water over a 50-day period.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to dicamba during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved dicamba. Calculations of exposure to dicamba in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to dicamba are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Dicamba Application

Available application data for dicamba use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to dicamba differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of dicamba in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Dicamba does not adsorb well to soil particles compared with other herbicides. Dicamba salts have a high solubility in water. The persistence of dicamba is markedly affected by rainfall, soil pH, and temperature. Dicamba is among the most mobile herbicides measured in soil.

4.2 Exposure

Estimates of potential exposure to dicamba are based on aerial as well as ground application. The maximum aerial application rate for dicamba (see Table 1-1) is 1.4 lb a.i./acre, while the compound

Table 4-1

USDA Forest Service Application Data for Dicamba (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Dicamba[b] (28)	Ground Foliar (water carrier)	3.5	98	5.9	13.6	7.3	18.0	Range (15) Noxious Weeds (12) Nursery (1)
Dicamba (4)	Ground Broadcast (pellets)	2.3	9	5.5	1.5	7.0	2.3	Noxious Weeds (2) Timber (1) Range (1)
Dicabmba[b] (1)	Aerial	7	7	4	4	6	6	Firebreak (1)
Dicamba (7)	Injection, cut surface	12.9	90	6	29.7	7.3	44	Timber (6) Wildlife (1)
Dicamba[b] (4)	Basal Spray (oil carrier)	5.8	23	5.8	36.3	7.3	43.8	Timber (2) Wildlife (2)

[a]Grand Total = 227

[b]Dicamba + 2,4-D

Source: Gross 1983

is applied from the ground (ground foliar) at rates as high as 6.0 lb a.i./acre. In both aerial application and ground foliar application, dicamba is applied as a liquid. The calculations in Table 4-2 that are based on aerial application (e.g., spray/observer dose, wildlife dermal exposures, and water concentrations) assume an application rate of 1.4 lb a.i./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose, and wildlife oral exposures) assume an application rate of 6.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of dicamba and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of dicamba to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational exposure estimates for dicamba are based on exposures on a per pound per acre application rate multiplied by 1.4 lb/acre for observers and 6.0 lb/acre for backpack sprayers. In the case of the aerial spray observer, dose estimates are based upon a of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent.

At 1.4 lb a.i./acre aerial application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: 0.056 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 6.0 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.24 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Table 4-2

Summary of Occupational Dose, Environmental Exposure, and Toxicity for Dicamba

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg		[b]
Observer	up to 0.056 mg/kg		
Backpack Sprayer	0.24 mg/kg		
Environmental Exposure			
Fish	0.014 ppm (Immersion)		[c]
Rabbits	0.564 mg/kg	Negligible	6.30 mg/kg
Deer	0.141 mg/kg	Negligible	8.64 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.00028 mg/kg (2 days)
(Rabbits)	NA	NA	0.039 mg/kg (2 days)
(Deer)	NA	NA	0.052 mg/kg (2 days)
(Water)	NA	NA	0.0006 mg/kg (1 day)
(Berries)	NA	NA	0.0014 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0007 mg/kg (1 day)
Toxicity Summary	LD_{50} >2,000 mg/kg in rabbits	LC_{50} >200 ppm in rats	Acute oral LD_{50} ≥566 mg/kg in rats, mice, guinea pigs, and rabbits. Chronic oral NOEL = 1,000 mg/kg in mice.
	Most studies, LC_{50} >50 ppm for fish		

[a]Assumes aerial application at 1.4 lb active ingredient per acre and ground foliar application at 6.0 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure.

[c]Fish exposure results from immersion in water containing dicamba.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by dicamba application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 1.4 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 0.5642 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.1414 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. This exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of dicamba in water, calculated as:

Water concentration: 0.014 ppm (0.01 ppm at 1.0 lb/acre aerial application rate)

This concentration of dicamba represents the estimated maximum concentration immediately after spraying.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of dicamba by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 6.0 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 8.64 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 6.30 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of dicamba in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.014 ppm

- bioconcentration: 1.0 (dicamba has a low tendency to bioaccumulate (See Appendix F)).

Fish tissue concentration = 0.014 ppm (0.014 mg/kg)

Deer and rabbit tissue concentrations include dicamba accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied dicamba is absorbed at a rate of 10 percent. It is also assumed that oral exposure of deer and rabbit to dicamba lasts only for 2 days due to the relatively short environmental persistence of dicamba. It was assumed that 15 percent of ingested dicamba would be absorbed, based

on limited metabolism data (refer to Appendix F). Deer tissue concentrations of dicamba are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.1414 mg/kg (dermal exposure) x 0.1 = 0.01414 mg/kg.

Oral Exposure contribution assumes 15 percent assimilation of two-days feeding, or 8.64 mg/kg/day (oral exposure) x 0.15 x 2 days = 2.592 mg/kg.

Total Deer Tissue concentration = 0.0141 mg/kg + 2.592 mg/kg = 2.60614 mg/kg (2.606 ppm).

Rabbit tissue concentrations of dicamba are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.5642 mg/kg (dermal exposure) x 0.1 = 0.05642 mg/kg.

Oral Exposure contribution assumes 15 percent absorption of two-days feeding, or 6.30 mg/kg/day (oral exposure) x 0.15 x 2 days = 1.890 mg/kg.

Total Rabbit Tissue Concentration = 0.05642 mg/kg + 1.890 mg/kg = 1.94642 mg/kg (1.946 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to dicamba from eating meat are derived:

Fish meat: 0.014 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.00028 mg/kg.

Deer meat: 2.606 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0521 mg/kg.

Rabbit meat: 1.946 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0389 mg/kg.

Water and plant consumption by man would result in the following oral exposures to dicamba based on assumptions stated in the General Introduction to the Herbicide Background Statements at an application rate of 1.4 lb a.i./acre (aerial):

Water: 0.0006 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries: 0.0014 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms: 0.0007 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of dicamba to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rat inhalation results, rabbit and rodent acute oral LD_{50}'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for Forest Service applications. Based upon chronic feeding for 19.5 months to determine carcinogenicity, a NOEL of 1000 mg/kg is reported in mice. Assuming a 100-fold margin of safety for extrapolation to man (a factor of 10 for inter-species variability and a factor of 10 for human intra-species variability), one can assume that 1,000 mg/kg 100, or a 10 mg/kg oral dose in man, will not result in adverse acute effects. The estimated human exposures from ingestion of water, game, and plant material are well below 10 mg/kg per day.

Small mammal studies have shown that dicamba applied to the eye is mildly irritating and that dicamba applied to the skin also is mildly irritating. Only a high dose (relative to those expected for humans) of dicamba in rabbits decreased the number of implantations and number of live fetuses. Dicamba is not mutagenic and does not appear to be carcinogenic. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from dicamba use, if proper care is taken during application.

Appendix A

Summary of Dicamba Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba	Honeybee (Insecta)	Ingestion	--	LD_{50} = 3.6 µg/bee	--[a]	Edson and Sanderson 1965 in Pimentel 1971
Dicamba		Ingestion	--	LD_{50} >10 µg/bee	Acetone solution of dicamba mixed with sucrose fed to bees.	Stevenson 1978
Dicamba	Honeybee (Apis mellifera; Insecta)	Ingestion	up to 60 days	No reduction in rate of mortality (half-life of colony) of bees fed 10, 100, and 1,000 ppm (weight) dicamba compared to controls.	Dicamba concentration in 60% sucrose solution.	Morton et al. 1972
Banvel®D4S	Honeybee (Apis mellifera; Insecta)	Ingestion	up to 60 days	No reduction in rate of mortality (half-life of colony) of bees fed 10, 100, and 1,000 ppm (weight) Banvel® compared to controls.	Banvel® concentration in 60% sucrose solution.	Morton et al. 1972
Dicamba	Honeybee (Apis mellifera; Insecta)	Surface absorption (contact)	--	LD_{50} >100 µg/bee	Unformulated dicamba dissolved in acetone applied to anesthetized bee.	Stevenson 1978 Stevenson 1978
Dicamba	Honeybees (Insecta)	Surface absorption (contact)	--	2.6 percent mortality in bees dusted with dose of 91 µg/bee.	--	Atkins 1975 in Ghassemi et al. 1981

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba	Cockroach (Blattella germanica; Insecta)	Ingestion	--	Dicamba at 1,000 ppm in food had no effect on larval mortality, development time of larvae, or reproductive potential of females through two generations compared to controls.	--	Riviere 1976
Dicamba	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption	48 hours	EC_{50} = 11 ppm	15.6°C	Sanders and Cope 1966 in Hulbert 1975
Dicamba, Technical (88%) Material	Decapod (shrimp) (Palaemonetes sp.; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} >56 ppm	Hard water, 21°C, mature specimens.	Johnson and Finley 1980
	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} >100 ppm	Hard water, 21°C, 1st instar specimens.	
	Sowbug, Isopod (Asellus sp.; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	Hard water, 15°C, mature specimens.	
	Amphipod (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} 100 ppm	Hard water, 15°C, mature specimens.	
Dicamba	Amphipod (Gammarus lacustris; Crustacea)	Surface absorption	96 hours	LC_{50} = 3.9 ppm	21.1°C	Sanders 1969 in Pimentel 1977
		Surface absorption	24 hours	LC_{50} = 10 ppm	--	
Dicamba	Amphipod (Gammarus lacustris; Crustacea)	Surface absorption	48 hours	LC_{50} = 5.8 ppm	--	U.S. Department of the Interior 1968 in Pimental 1971

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba	Seed shrimp, Decapod (Cypridopsis vidua; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	21.0°C	Cope 1970
	Scud, Amphipod (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	15.5°C	
Dicamba	Sowbug, Isopod (Asellus brevicaudus; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	15.5°C	
Dicamba	Glass shrimp, Decapod (Palaemonetes kadiakensis; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	21.0°C	Cope 1970
	Crayfish, Decapod (Oronectes nais; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	15.5°C	
Banvel®, Technical	Cladoceran (Daphnia sp.; Crustacea)	--	48 hours	LC_{50} = 111 ppm	--	Velsicol Chemical Corporation data cited in Ghassemi et al. 1981
Banvel®, Technical (86.8% a.i.)	Glass shrimp (Crustacea)	--	96 hours	LC_{50} >100 ppm	--	Velsicol Chemical Corporation data and U.S. Environmental Protection Agency data cited in Ghassemi et al. 1981
	Fiddler crab (Crustacea)	--	96 hours	LC_{50} >180 ppm	--	

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba (Sodium salt)	Bacteria	Surface absorption	7 days	It was reported that 60% (sic) of 50 cultures showed reduced or no growth with 50 ppm herbicide in medium.	--	Cooper et al. 1978
Dicamba (Sodium salt)	Actinomycetes	Surface absorption	7 days	It was reported that 14% (sic) of 40 cultures showed reduced or no growth with 50 ppm herbicide in medium.	--	Cooper et al. 1978
Dicamba (Sodium salt)	Fungi	Surface absorption	7 days	It was reported that 6% (sic) of 70 cultures showed reduced or no growth with 50 ppm herbicide in medium.	--	Cooper et al. 1978
Dicamba (Sodium salt)	Yeasts	Surface absorption	7 days	None of 15 cultures showed reduced or no growth with 50 ppm herbicide in medium.	--	Cooper et al. 1978
Dicamba	17 Strains of Algae (Chlorophyceae)	Herbicide incorporated into culture medium.	--	Two of 17 strains showed reduced growth with dicamba up to 10 ppm.	--	Cullimore 1975
Dicamba	Soil Microbial Populations	Herbicide incubated in soil at a concentration equivalent to 5 tons per acre.	56 days	CO_2 evolution reduced by 11 to 14% compared to controls.	Calcareous-loam soil.	Stojanovic et al. 1972

[a]Data not available or not applicable.

Appendix B

Summary of Dicamba Toxicity Data for Fish and Amphibians

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba (liquid)	Bluegill	Surface absorption	24 hours 48 hours	LC_{50} = 600 ppm LC_{50} = 410 ppm	--[a]	Hughes and Davis 1962 in Lorz et al. 1979
Dicamba (Banvel D®, acid)	Bluegill	Surface absorption	24 hours	LC_{50} = 20 ppm	Banvel D® adsorbed onto vermiculite.	
Dicamba, Technical (88% Material)	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 28 ppm	Temperature 12°C, 0.8 gram fingerlings.	Johnson and Finley 1980
	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} > 50 ppm	Temperature 12°C, 0.8 gram fingerlings.	
Dicamba	Coho salmon	Surface absorption, static bioassay	24 hours 48 hours	LC_{50} = 151 ppm (a.i.) LC_{50} = 120 ppm (a.i.)	Juveniles, pH about 7.7.	Bond et al. 1965 in Lorz et al. 1979
Dicamba (Banvel D®)	Rainbow trout	Surface absorption	48 hours	LC_{50} = 35 ppm	Values were reported as "estimated 48-hour LC_{50}s".	Bohmont 1967 in Pimentel 1971
	Bluegill	Surface absorption	48 hours	LC_{50} = 130 ppm		
Dicamba (Banex®)	Mosquito fish	Surface absorption, static bioassay	24 hours 48 hours 96 hours	TL_{50} = 516 ppm TL_{50} = 510 ppm TL_{50} = 465 ppm	21 to 22°C	Johnson 1978
Dicamba	Rainbow trout	Surface absorption	72 hours	100 percent morbidity at 320 ppm	--	Bond et al. 1965 in Lorz et al. 1979
Dicamba (Banvel®)	Coho salmon	Surface absorption, static bioassay	144 hours	No effect observed at concentrations up to 100 ppm	Yearling salmon. Dissolved oxygen 8.5 ppm, pH 7.03, temperature 10±1°C.	Lorz et al. 1979

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba (Technical)	Bluegill	Surface absorption	48 hours	LC_{50} = 40 ppm	24°C	Cope 1965 in Sanders 1970
Banvel® (4 lb/gal, DMA)	Bluegill	Surface absorption	96 hours	LC_{50} >1,000 ppm	--	Velsicol Chemical Corporation data and EPA registration files cited in Ghassemi et al. 1981
Banvel® (Technical 86.8% a.i.)	Sheephead minnow	Surface absorption	96 hours	LC_{50} >180 ppm	--	
Dicamba (Technical, 88%)	Cutthroat trout,	Surface absorption, static bioassay	96 hours	LC_{50} >50 ppm (a.i.)	When dicamba at 50 ppm was tested in combination with 2,4-D esters (butyl ester and propylene glycol butyl ether ester) and picloram, there was no synergistic effect on dicamba or the other herbicides.	Woodward 1982
Banvel® (Technical)	Bluegill	Surface absorption	96 hours	LC_{50} = 135 ppm	--	Velsicol Chemical Corporation data cited in Ghassemi et al. 1981
	Rainbow trout	Surface absorption	96 hours	LC_{50} = 135 ppm	--	
Dicamba (Banex®)	Frog, *Adelotus brevis*	Surface absorption	24 hours 48 hours 96 hours	LC_{50} = 220 ppm LC_{50} = 202 ppm LC_{50} = 185 ppm	One to 2-week old tadpoles, water temperature 21 to 22°C.	Johnson 1976
	Frog, *Limnodynastes peroni*	Surface absorption	24 hours 48 hours 96 hours	LC_{50} = 205 ppm LC_{50} = 166 ppm LC_{50} = 106 ppm		

[a] Data are not available or not applicable.

Appendix C

Summary of Dicamba Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Dicamba	Pheasants	Assumed to be oral.	--[a]	LD_{50} = 673 mg/kg (females) LD50 = 800 mg//kg (males)	--	Edson and Sanderson 1965 in Pimentel 1971
Banvel 310® (Technical)	Mallard duck	Ingestion	Acute	LC_{50} = 2,000 mg/kg	--	EPA registration data cited in Ghassemi et al. 1981
Banvel® (Technical 86.8% a.i.)	Mallard duck	Ingestion	Acute	LC_{50} = 2,000 mg/kg	--	Velsicol Chemical Corporation data cited in Ghassemi et al. 1981
	Mallard duck	Ingestion	8 days	LD_{50} >10,000 ppm	--	
	Bobwhite quail	Ingestion	8 days	LC_{50} >10,000 ppm	--	
Banvel XP® (10% a.i.)	Mallard duck	Ingestion	8 days	LC_{50} >10,000 ppm	--	EPA registration data cited in Ghassemi et al. 1981
	Bobwhite quail	Ingestion	8 days	LC_{50} >10,000 ppm	--	
Banvel 310® (4 lb/gal. dimethylamine salt)	Mallard duck	Ingestion	8 days	LC_{50} >4,600 ppm	--	Velsicol Chemical Corporation data cited in Ghassemi et al. 1981
Dicamba	Chicken (eggs)	Injection	--	Decreased rate of hatching compared to controls at doses giving a concentration in the egg of 400 ppm. No effect at 300 ppm, but an anomalous low hatch of 200 ppm due to early mortality.	No teratogenic effects observed. Acetone solution used as a carrier.	Dunachie and Fletcher 1970

[a]Data are not available or not applicable.

Appendix D

Summary of Dicamba Toxicity in Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Dicamba	Guinea pig	Single dose-oral	Oral LD_{50} was 566 mg/kg	--[a]	Edson and Sanderson 1975 in Hayes 1982 and Golovan 1970 in Hayes 1982
			Oral LD_{50} was 3,000 mg/kg		
Dicamba	Mouse (female)	Single dose-oral	Oral LD_{50} was 1,189 mg/kg	--	Golovan 1970 in Hayes 1982
	Mouse (male)		Oral LD_{50} was 1,190 mg/kg	--	
Dicamba, acid	Mouse		Oral LD_{50} > 4,600 mg/kg	One measurement.	U.S. Department of Energy 1983
Dicamba	Rabbit	Single dose-oral	Oral LD_{50} was 566 mg/kg	--	Edson and Sanderson 1965 in Hayes 1982 and Golovan 1970 in Hayes 1982
			Oral LD_{50} was 2,000 mg/kg		
Dicamba	Rat (male)	Single dose-oral	Oral LD_{50} was 757 mg/kg	--	Edson and Sanderson 1965 in Hayes 1982
	Rat (female)		Oral LD_{50} was 1,414 mg/kg		
Banvel®, Technical	Rat	--	Oral LD_{50} was 1,707 to 2,900 mg/kg	--	Velsicol Chemical Corporation 1979 in Ghassemi et al. 1981
Banvel®, 4 lbs/gal. DMA	Rat	--	Oral LD_{50} was 1,028 to 2,629 mg/kg	--	
Dicamba, DMA salt	Rat	--	Oral LD_{50} was 1,028 mg/kg	--	U.S. Department of Energy 1983
Dicamba, acid	Rat	--	Oral LD_{50} was 2,900 $\pm$ 800 mg/kg	--	Weed Science Society of America 1983
Banvel D®	Sheep	10 daily doses of 250 mg/kg; route not specified	No adverse effect.	--	Palmer and Radeleff 1964

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Concluded)		
Banvel D®	Sheep	2 doses of 500 mg/kg; route not specified	Fatal to the one sheep treated.	--	Palmer and Radeleff 1964
Banvel D®	Sheep	1 dose of 1,000 mg/kg; route not specified	No adverse effect.	--	
Banvel D®	Sheep	5 doses of 250 mg/kg; route not specified	No adverse effect.	--	
			Acute Intraperitoneal Toxicity		
Dicamba	Rat (male)	Intraperitoneal	LD_{50} was 80 mg/kg	--	Edson and Sanderson 1965 in Hayes 1982
			Acute Dermal Toxicity		
Banvel®, Technical	Rabbit	Dermal	Dermal LD_{50} >2,000 mg/kg	--	Velsicol Chemical Corporation 1979 in Ghassemi et al. 1981
Banvel®, 4 lbs/gal. DMA	Rabbit	Dermal	Dermal LD_{50} >2,000 mg/kg	--	
Dicamba	Rabbit	Dermal	Skin irritation with extended contact at all doses up to 2,500 mg/kg/day	No other signs of toxicity; 7 of 32 rabbits died due to nontreatment related causes.	U.S. Department of Energy 1982
Dicamba	Rabbit	Dermal	Dermal sensitization followed 3 weeks of application of dicamba 3 x weekly. The animals were challenged 14 and 16 days following the last dose.	Dicamba has moderate sensitization potential.	U.S. Department of Energy 1982

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Concluded)		
Dicamba, DMA salt	Rabbit	Applied undiluted daily for 2 weeks	Very mild irritation.	--	Weed Science Society of America 1983
Dicamba, DMA salt	Rabbit	Applied 1:40 dilution in water daily for 30 days	No irritation.	--	
Dicamba, DMA salt	Rat	Applied undiluted daily for 2 weeks	Very mild irritation.	--	
Dicamba, DMA salt	Rat	Applied 1:40 dilution in water for 30 days	No irritation.	--	
			Eye Irritation		
Dicamba, DMA salt	Rat & Rabbit	0.1 ml of aqueous solution	No injury to cornea or iris.	Low grade irritation disappeared rapidly.	Velsicol 1971 in U.S. Department of Agriculture 1981
Dicamba, DMA salt	Rat & Rabbit	0.2% to 2.0% administered as single doses or as repeated doses for 1 week	No irritation or injury.	--	
			Inhalation Toxicity		
Banvel® DMA Technical	Rat	Inhalation	Inhalation LC_{50}; 4-hour exposure; >200 ppm	--	Velsicol Chemical Corporation 1979 in Ghassemi et al. 1981
Banvel 310®, 4 lbs/gal.	Rat	Inhalation	Inhalation LC_{50}; 4-hour exposure; >200 ppm		
Banvel®, Technical	Not reported	Inhalation	LC_{50} 4-hour dust was >9.6 ppm	--	U.S. Department of Energy 1983
Banvel D®, Technical	Not reported	Inhalation	LC_{50} 4-hour dust was >200 ppm	--	

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity		
Dicamba	Rat (1 male and 1 female per dose)	3 week dietary study; dosages: 658 to 23,500 ppm	No evidence of toxicity.	--	U.S. Department of Energy 1983
Dicamba	Rat (5 male and 5 female per dose)	4 week dietary study; dosages: 5,000, 7,500, 10,000, 12,500, and 15,000 ppm	Weight gain was decreased at high dietary levels. Posterior weakness developed in 7 of 10 rats at 15,000 ppm and in 1/10 rats at 12,500 ppm.	--	
Dicamba, acid	Rat	800 ppm in diet for 3 months	No adverse effect.	--	Weed Science Society of America 1983
Dicamba, Technical	Rat	13 week dietary study; dosages: 5,000 ppm and 10,000 ppm	10,000 ppm produced histological changes in liver. The number of cytoplasmic vacuoles in liver parenchyma was reduced.	5,000 ppm suggested to be the "no effect" dietary level.	U.S. Department of Energy 1983
Dicamba	Rat (male and female)	13 week dietary study dosages: 100, 500, 800, 1,000 ppm	Food consumption and growth rate normal; no deaths; no pathology at 7 weeks but at 13 weeks liver and kidney pathology were observed in 800 and 1,000 ppm and not 100 and 500 ppm groups.	--	U.S. Department of Agriculture 1981
			Teratology, Reproduction and Fertility		
Dicamba, Technical	Rabbit	Dosages: 1, 3, 10 or 20 mg/kg/day on days 6 to 18 of gestation	Post-implanation losses and decreased number of live fetuses were recorded at 10 and 20 mg/kg/day. No effect at 3 mg/kg/day. A nontreatment related decrease in post-implantation loss was observed in 1 mg/kg/day group.	No evidence for teratogenicity at 10 mg/kg/day or lower doses. At 10 mg/kg/day observed decreased total weight and post-implantation losses which were not considered to be teratogenic.	U.S. Department of Energy 1983

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Teratology, Reproduction and Fertility (Concluded)		
2-Methoxy-5-hydroxy-3,6-dichlorobenzoic acid	Rat and Dog	100 and 250 ppm in diet for 90 days	No adverse effects.	--	U.S. Department of Energy 1983
Dicamba	Rat	2 males and 4 females fed 500 ppm dicamba for 3 to 4 months then were mated. Also, 2 treated males and 4 treated females were mated with untreated rats.	No change in reproductive capacity in parents or offspring were observed.	--	Velsicol 1971 in U.S. Department of Energy 1983
Dicamba	Rat	In a three generation reproduction study 500 ppm dicamba were provided in the diet for 3 to 4 months; use second litter of each generation.	No changes in fertility, gestation, viability or lactation in any group were observed.	--	U.S. Department of Energy 1983
			Carcinogenicity		
Dicamba	Dog, Beagle	2 year dietary study study; dosages: 5, 50 ppm	No carcinogenicity reported.	No effect on survival, body weight, food consumption, 0.1 gram weight, hematology, or histology of viscera.	Weed Science Society of America 1983

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Concluded)		
Dicamba	Mice	Dietary administration of 100, 1,000, and 10,000 ppm dicamba for 14 to 19.5 months	No carcinogenicity reported.	At 10,000 ppm observed decreased body weight and increased liver weight. Enlargement of liver cells was only dose-dependent effected. Due to increase mortality, the 10,000 ppm dose group was terminated at 19.5 months.	U.S Department of Energy 1983
Dicamba	Rat	2 year dietary study; dosages: 5, 50, 100, 250, and 500 ppm		No measurable effect on survival, body weight, food consumption, organ weight, hematology, or histology of viscera. No tissue storage. Rapid urinary excretion.	Weed Science Society of America 1983

[a]Data are not available or not applicable.

Appendix E

Summary of Dicamba Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Dicamba	Mice, (male)	1,000 mg/kg orally or 30 mg/kg intrapertioneally	Negative: no early embryonic death following weekly matings with fertile females.	Dominant lethel test.	U.S. Department of Energy 1983
Dicamba	Bacillus subtilis	1 mg/ml	Negative	Recombination assay.	
Dicamba	Not specified	300 mg/kg, route not specified	Negative	Host mediated assay.	Velsicol 1981 in Newton and Dost 1981
Dicamba	Salmonella typhimurium	Dose not specified; Strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence and absence of S9	Nonmutagenic	Histidine reverse mutation assay.	Poole et al. 1977; Eisenbeis et al. 1981; and Anderson et al. 1972
Dicamba	Escherichia coli WP2	Dose not specified. In the presence and absence of liver activation system	Nonmutagenic	Tryptophan mutation system assay.	Poole et al. 1977
Dicamba	Saccharomyces cerevisiae D3	Dose not specified. In the presence and absence of liver activation system	Nonmutagenic	Mitotic recombination assay.	
Dicamba	WI-38 (human fibroblasts)	Dose not specified	Negative	Unscheduled DNA synthesis assay.	Poole et al. 1977
Dicamba	E. coli (W3110 P3478)	Not specified	Positive: for preferential toxicity to repair-deficient strains.	Relative toxicity assays. Tested DNA repair proficient (W3110 and H17) and DNA repair-deficient (P3470 and M47) strains.	
Dicamba	B. subtilis (H17 and M45)				
Dicamba	E. coli Bacteriophage T_4 AP72 and rII mutants	Not specificed	Negative	Induction of rII mutants of T_4 bacteriophage reversion of bacteriophage AP72 to T_4.	Andersen et al. 1972

Appendix F

Summary of Metabolism Studies with Dicamba

Formulation	Organism	Dose	Comments	References
Dicamba (^{14}C)	Cow (lactating)	2.2 mg/kg body weight per day for 5.5 days (equivalent to 60 ppm in feed). Total of 4.95 grams.	Of the total administered dose of dicamba, 88.8% was excreted in urine, 1.5% in feces, and 0.018% in milk. Urine measurements were within 6 hours of final dose. Maximum concentrations of dicamba were 0.04 ppm in milk. At autopsy, maximum tissue levels were: 2.59 ppm in kidney; 0.40 ppm in blood; 0.30 ppm in liver; and 0.25 ppm in ovary. Muscle tissue had 0.02 to 0.03 ppm residues. Most dicamba (75 to 84%) was eliminated unchanged, some (8 to 18%) was metabolized to 3,6-dichloro-2-hydroxybenzoic acid.	Oehler and Ivie 1980
Dicamba	Lactating dairy cattle	9 day dietary study; dosages: 10, 25, 50 80 and 400 ppm	No residue in milk at 10 to 50 ppm levels. Residues not exceeding 0.15 ppm in milk after 9 days of 30 and 400 ppm doses.	Velsicol 1971 in U.S. Department of Agriculture 1981
Dicamba (^{14}C)	Various	5 mg applied to sorghum in a model ecosystem corresponding to an application rate of 1 lb/acre (1.12 kg/ha).	Residues of dicamba and its metabolites did not accumulate in organisms in the model ecosystem. Algae had the highest residue levels at 1.62 ppm, while fish, at the top of the feed chain, contained tissue levels of only 0.02 ppm.	Sanborn 1974; Yu et al. 1975

Appendix F (Concluded)

Formulation	Organism	Dose	Comments	References
Dicamba (^{14}C)	Rat	0.1 to 0.93 g/kg body weight administered by esophageal intubation, incorporation in in feed, and subcutaneous injection.	More than 90 percent of dose eliminated in urine regardless of route to administration, feces contained 0.8 to 7.0% of dose. Highest residue levels in liver and kidney; lowest in blood, muscle and fat. Tissue levels proportional to dose. Most of the ^{14}C activity in the form of ^{14}C parent dicamba, at dietary levels of 1,000 ppm, at 20% of urinary residues were conjugated with glucuronic acid.	Tye and Engel 1967 in Paulson 1975 and in Hayes 1982
Dicamba	Cow	--[a]	Seventy-three percent of total dose detected unchanged in urine within 7 days. None detected in feces or milk.	St. John and Lisk 1969 in Hayes 1982
Dicamba (^{14}C)	Earthworm (Lumbricus terrestris)	Earthworms taken from untreated control plots and from plots treated for 10 years with dicamba at 0.25 lb/acre (0.28 kg/ha). Worms were injected with 10 μg/g.	Worms taken from plots previously treated with dicamba metabolized more, ^{14}C labeled dicamba in the laboratory than worms from untreated control plots.	Chio and Sanborn 1978
Dicamba	Man	--	Based on almost 7,000 blood samples from the general population, 1.2% were positive for dicamba. The high value was 58 ppb.	Kutz 1983

[a]Data are not available or not applicable.

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Herbicide Background Statement: Fosamine Ammonium

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Fosamine Ammonium Summary

Major Trade Name: Krenite®

Chemical Name: Ammonium ethyl carbamoylphosphonate

Major Forestry Applications: Applied as a selective herbicide for conifer release, right-of-way maintenance, noxious weed control, engineering uses, wildlife habitat improvement, and range management.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Fosamine ammonium has a very low toxicity to those invertebrates in which it has been determined, with LC_{50}'s in excess of 1,000 ppm. Soil fungi and bacteria are unaffected by fosamine ammonium at concentrations of 100 ppm, but algae exhibited inhibition of nitrogen fixation at 100 ppm. Fosamine ammonium is practically nontoxic to fish, with LC_{50}'s greater than 100 ppm in all species tested. However, sublethal effects, such as avoidance, were seen in salmon at 8.9 ppm. In birds, fosamine ammonium has a low toxicity, with LD_{50}'s >5,000 mg/kg in those species tested.

In experimental mammals, fosamine ammonium without surfactant was not an eye irritant, but with surfactant was an irritant to the iris and conjunctiva. Fosamine ammonium is a mild to moderate skin irritant. Chronic feeding in rats resulted in no toxic effects and no birth defects. In four of five *in vitro* tests, fosamine ammonium was not mutagenic. Based on toxicity exposure and hazard assessment evaluations, the estimated human exposures by dermal, inhalation, and oral routes are well below the estimates of toxicities in mammals.

Environmental Fate

Fosamine ammonium is absorbed by plant stems, buds, and foliage. Although it is translocated within the plant, effective action requires the complete coverage of all parts of woody plants. Fosamine ammonimum has a short persistence in plants and is metabolized to products which, in turn, have a relatively short persistence. It does not bioaccumulate in animals, and once absorbed, is eliminated rapidly in feces and urine as a metabolite or as the parent compound.

In soil, fosamine ammonimum has a very short persistence (half-life of 1 week to 10 days in research studies), is strongly adsorbed to soil particles, and is not mobile. It is rapidly degraded by microorganisms. In water, loss of activity of fosamine ammonimum is by microbial decomposition, adsorption onto sediments, and dilution.

Exposure and Hazard

Estimated occupational and environmental exposures to fosamine ammonimum from normal use are below those levels that would be expected to have acute or chronic toxic effects or reproductive effects. Fosamine ammonium is nonmutagenic in four of five systems tested. No carcinogenicity data are available.

1.0 General Information

Fosamine ammonium, also known by the trade name* Krenite®, is a herbicide and plant growth regulator that is generally used for selective woody plant control. Its mode of action in plants is to inhibit or prevent bud development. It is absorbed by buds, stems, and foliage of plants (Weed Science Society of America 1983).

1.1 Normal Use Pattern

Fosamine ammonium has been available commercially for only the past few years since its phytotoxic properties were first reported in 1974 (Han 1979a in Ghassemi et al. 1981). It is registered for control and growth suppression applications in blackberry, white oak, water oak, red oak, loblolly and Virginia pine, sweet gum, sumac, black locust, salmonberry, thimbleberry, vine maple, American elder, eastern white pine, multiflora rose, bracken fern, Eastern cottonwood, sycamore, winged elm, slippery elm, tree-of-heaven, wild grape and plum, and quaking aspen. It is also registered for contol of field bindweed and for partial control and growth suppression of species such as red alder, hawthorn, wild cherry, maple, white ash, black gum, birch, black cherry, pincherry, basswood, hickory, willow, sassafras, yellow poplar, elm, big leaf maple, choke cherry, persimmon, red maple, sourwood, and tulip tree (Weed Science Society of America 1983). In noncrop areas, fosamine ammonium is used along rights-of-way to maintain railroad, utility, pipeline, and highway corridors; along drainage-ditch banks; at industrial plant sites and storage areas; and on land areas to be cleared adjacent to and surrounding domestic water supply reservoirs, supply stations, lakes, and ponds (Meister Publishing Company 1981).

Fosamine ammonium is used in forestry for conifer release, rights-of-way maintenance, noxious weed control, engineering uses, wildlife habitat improvement, and range management. Typical application rates are from 6 to 12 lb a.i./acre (6.8 to 13.5 kg/ha), with the higher rates to be used on brush stands and on areas where only resistant species predominate. Water is the usual carrier for application of fosamine ammonium at a rate of 50 to 300 gallons per acre for ground spray and 10 to 40 gallons per acre for aerial spray. Nonionic surfactants are usually added to the spray mixture to increase the rate of penetration into the plant (Weed Science Society of America 1983). Statistics on the use of fosamine ammonium by the USDA Forest Service are presented in Table 1-1. Application rates ranged from 0.25 to 8.0 lb a.i. a.i./acre for the ten USDA Forest Service projects in which fosamine ammonium was used in 1982.

*Trade names are used only to provide information and do not imply product endorsement.

TABLE 1-1

USDA Forest Service Fosamine Ammonium use

Purpose	Active Ingredient per Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
1981					
Aerial					
Conifer release	4.3	4.3	222	Liquid	Helicopter
Right-of-way	10.0-10.7	10.3	91	Liquid	Helicopter
Ground					
Noxious weeds	0.6	0.6	50	Liquid	Individual plant
Right-of-way	1.2-8.7	3.2	498	Liquid	Broadcast
Site preparation	11.5	11.5	96	Liquid	Broadcast
Wildlife habitat improvement	1.5	1.5	45	Liquid	Individual plant
1982					
Ground (10 Projects)					
Engineering, Wildlife, and, Conifer Release	0.25-8.0	3.4	1,530	Liquid	Ground foliar

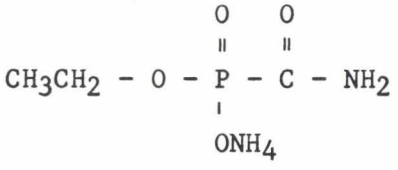
Source: Gross 1983.

Fosamine ammonium is manufactured and marketed in the United States by the E.I. DuPont de Nemours Company under the trade names of Krenite® Brush Control Agent and Krenite®S Brush Control Agent which are available as liquid formulations (Meister Publishing Company 1981). Table 1-2 lists the major formulations of fosamine ammonium marketed in the United States.

Fosamine ammonium is best applied in the late summer or early fall, before autumn coloration. It is absorbed by plant foliage, stems, and buds with no immediate visible effect in deciduous species and with a browning of foliage soon after application in many nondeciduous species. In the following spring, after treatment, bud development is either totally suppressed, resulting in no formation of new foliage, or inhibited so as to form small, spindly leaves. In many cases, the stems of woody plants may remain alive for several years after fosamine ammonium application (Weed Science Society of America 1983 and Ghassemi et al. 1981).

1.2 Physical and Chemical Properties

The active ingredient of fosamine ammonium and its formulations is ammonium ethyl carbamoylphosphonate, with the following structural formula (Dupont 1983):



Fosamine ammonium has a molecular formula of $C_3H_{11}N_2O_4P$ and a molecular weight of 170.1. In its pure form, it is a white crystalline solid with a melting point of 347°F (175°C) and a specific gravity of 1.33. At approximately 77°F (25°C), it is highly soluble in water (179 grams/100 grams water), moderately soluble in methanol (15.8 grams/100 grams methanol), and only slightly soluble in other organic solvents (0.004 grams/100 grams chloroform; 0.02 grams/100 grams n-hexane; 0.03 grams/100 grams acetone; 0.04 grams/100 grams benzene; 0.14 grams/100 grams dimethylformamide; and 1.20 grams/100 grams ethanol) (Dupont 1983).

Fosamine ammonium is corrosive to brass and copper. Consequently, spray equipment should be thoroughly rinsed of all traces of fosamine ammonium after use (Weed Science Society of America 1983). At 77°F (25°C), the vapor pressure of fosamine ammonium is 4×10^{-6} mm Hg.

Table 1-2

Fosamine Ammonium Formulations

Formulation	Active Ingredients	Comments
Krenite® Brush Control Agent	41.5% fosamine ammonia (4 lb/gal. [480 g/ℓ])	Miscible liquid
Krenite® S Brush Control Agent	41.5% fosamine ammonia (4 lb/gal. [480 g/ℓ])	Miscible liquid, includes a surfactant.

SOURCE: Weed Science Society of America 1983.

2.0 Toxicity of Fosamine Ammonium

There are very few reports of animal studies on fosamine ammonium toxicity available in the open literature. Most studies have been conducted by E.I. DuPont de Nemours Company, the manufacturer of fosamine ammonium. This section draws, to a large extent, upon material provided by DuPont (Schneider and Kaplan 1983).

The toxicological properties of fosamine ammonium, summarized in Table 2-1, show that this herbicide is generally nontoxic or only very slightly toxic to those animal species tested. Fosamine ammonium is relatively toxic to a wide range of nontarget plant species, and even moderately susceptible and resistant plants will have some suppression of terminal growth as a result of contact with fosamine ammonium (Weed Science Society of America 1983). Studies with rodents, rabbits, dogs, and sheep, as well as laboratory cellular studies, indicate that fosamine ammonium is nonteratogenic, does not adversely affect reproduction, and is nonmutagenic. Carcinogenicity studies have not been reported for fosamine ammonium.

2.1 Invertebrates and Microorganisms

Fosamine ammonium toxicity has been determined for only a very few invertebrates and for a few microorganisms. The available studies are summarized in Appendix A and indicate that fosamine ammonium has a very low toxicity to those organisms tested, with LC_{50}'s in excess of 1,000 ppm in invertebrates and no significant effects on soil fungi and bacteria at concentrations as high as 100 ppm. However, algae exhibited inhibition of nitrogen fixation within 8 hours when treated with 100 ppm fosamine ammonium (Hallborn and Bergman 1979 in Ghassemi et al. 1981 and 1982). Toxic effects occurring at concentrations greater than 10 ppm in aquatic organisms are considered indicative of compounds that are only slightly toxic (Clarke et al. 1970).

For the most part, toxicity studies with invertebrates and microorganisms have been done in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of fosamine ammonium in a natural environment. For example, in the laboratory, aquatic organisms were kept up to 4 days with continuous exposure to fosamine ammonium. Microorganisms were grown in culture media containing fosamine ammonium, often at concentrations several orders of magnitude greater than those normally found in the environment following application. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to fosamine ammonium or other man-made chemicals is usually a transient phenomenon whose duration depends on the rate of streamflow and on the rate of decomposition of the chemical. Additionally, in natural

Table 2-1

Summary of Toxicological Properties of Fosamine[a]

1. Acute Oral Toxicity

Organism (Formulation)	Adverse Effects
Rat (Krenite®)	LD_{50} = 24,400 mg/kg
Rat (Krenite®S 41.5% a.i. + surfactant)	LD_{50} >5,000 mg/kg
Guinea pig (Krenite®)	LD_{50} = 7,380 mg/kg
Dog (42.5% a.i.)	ALD >12,800 mg/kg
Fish, adult (Fosamine Ammonium, 42% formulation)	LC_{50} >1,000 ppm
Birds (Fosamine Ammonium salt, unformulated)	LD_{50} >5,000 mg/kg

2. Acute Dermal Toxicity

Organism (Formulation)	Adverse Effects
Rabbit (41.1% a.i. + surfactant, 42% a.i.)	LD_{50} >1,683 mg/kg
Guinea pig (unformulated)	Not a skin irritant or sensitizer. Transient mild erythema.

3. Eye Irritation

Organism (Formulation)	Adverse Effects
Rabbit (Krenite® 42% a.i.)	Not an eye irritant.
Rabbit (41.1% a.i. + surfactant)	Transient corneal opacity and irritation to conjunctiva and iris.

Table 2-1 (Continued)

4. Inhalation Toxicity

Organism (Formulation)	Adverse Effects
Rat (Krenite®)	1 hour ALC >56.6 mg/l in males and >42.0 mg/l in females.
Rat (41.1% a.i. + surfactant)	Males: LC_{50} = 3.30 mg/l (95% c.l. = 2.57 to 4.48 mg/l) Females: LC_{50} = 2.75 mg/l (95% c.l. = 1.93 to 3.36 mg/l)

5. Intraperitoneal Toxicity

Organism (Formulation)	Adverse Effects
Rat (42% a.i.)	LD_{50} = 3,000 mg/kg

6. Subchronic Oral Toxicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (42% Formulation)	10 doses of 2,200 mg/kg/day for 2 weeks	No histopathological evidence of toxicity; mild diarrhea
Rat (unformulated)	0 to 5,000/10,000[b] ppm in diet for 90 days	No compound related toxicity swelling. NOEL = 5,000/ 10,000 ppm
Rat (unformulated)	1,000 ppm in diet for 3 months	No adverse effects except diarrhea.
Dog (unformulated)	0 to 10,000 ppm in diet for 6 months	Increased stomach weights in high dose females. NOEL = 1,000 ppm.
Sheep (41.5% a.i.)	0 to 2,000/2,500[b] ppm in diet for 90 days	NOEL = 2,000/ 2,500 ppm.

Table 2-1 (Concluded)

7. Reproduction, Teratology and Fertility

Organism	Dose and Length of Treatment	Adverse Effects
Rat (unformulated)	Fed 0 to 5,000/10,000 ppm through maturation, production and weaning of 2 litters	No adverse effect on reproduction or lactation. NOEL = 5,000/10,000 ppm.
Rat (42% a.i.)	Fed 0 to 10,000 ppm days 6 through 15 of gestation	No embryo-fetal toxicity or teratogenicity. NOEL = 10,000 ppm.

8. Mutagenicity

Organism (Formulation)	Adverse Effects
Salmonella typhimurium	Nonmutagenic
Chinese Hamster Ovary cells Point Mutation Assay	Nonmutagenic
Chinese Hamster Ovary cell In vitro Cytogenetic Assay	Chromosome damage with and without activation.
In Vivo Bone Marrow Cytogenic Assay in Rats	No chromosome aberrations.
Unschedule DNA Synthesis assay	Not induced.

[a]Toxicology data were extracted from Appendices A through E. Source references an be found in the appropriate appendix.
[b]This nomenclature means animals were initially placed on the low concentration diets, but due to an absence of toxicity the dosage in the diet was increased to the higher concentration.

systems, interaction with other man-made and naturally occurring chemicals and changes in environmental factors may affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions, in turn, potentially alter the tolerance levels of organisms to the herbicides.

All references to invertebrate toxicity studies with fosamine ammonium (Ghassemi et al. 1981 and 1982) are to two studies reported by DuPont (Schneider and Kaplan 1983) for honeybees and Daphnia (waterfleas) which showed 48-hour LC_{50}'s greater than 10,000 ppm and equal to 1,524 ppm, respectively. Three additional DuPont studies, one with soil bacteria on two soil types, a second with soil bacteria and fungi on three soil types, and a third with fungi in an agar plate assay, indicate no effect with fosamine ammonium concentrations as high as 20 ppm (for 5 weeks), 10 ppm (for 8 weeks), and 100 ppm, respectively. Hallborn and Bergman (1979 in Ghassemi et al. 1981 and 1982) reported drastic reduction in the rate of nitrogen fixation by algae (Nostoc sp.) at 100 ppm fosamine ammonium after 8 hours exposure.

2.2 Fish

The toxic effects of fosamine ammonium on fish are summarized in Appendix B. With the exception of a 96-hour LC_{50} of 670 ppm for adult bluegill sunfish, reported adult fish LC_{50}'s are all in excess of 1,000 ppm (Schneider and Kaplan 1983). Clarke et al. (1970) suggest that pesticides with median lethal concentrations of 10 ppm or more for fish be considered "slightly toxic." The alevin (yolk-sac fry) stage in salmonids (Coho salmon and rainbow trout) were the most sensitive to toxic levels of fosamine ammonium with LC_{50}'s of 618 and 367 ppm, respectively (McLeay and Gordon 1980 in Norris et al. 1983). Thus fosamine ammonium should be considered slightly toxic to nontoxic to fish.

Despite the relatively high lethal and sublethal toxic effect levels for fosamine ammonium, DuPont studies indicate that fish can detect fosamine ammonium at concentrations well below LC_{50} values and even below concentrations at which deaths are not observed. Coho salmon, with a 96-hour LC_{50} of 9,812 ppm (24-hour renewal), displayed a threshold of 8.9 ppm for avoidance responses and a threshold of 4 ppm for acute stress as measured by leucocrit values* (Schneider and Kaplan 1983). Schneider and Kaplan (1983) report that threshold-effect concentrations of Krenite® found for salmonids in partial life-cycle studies are less than 75 times the maximum theoretical concentration of Krenite® that would be found in shallow waters due to direct overhead spray application.

*Leucocrit measurements determine the amount of white blood cells (leucocytes) in the fish blood as a determinant of stress.

2.3 Birds

Acute toxicity and dietary studies with birds (mallard duck and bobwhite quail) conducted by E.I. DuPont de Nemours Company (Schneider and Kaplan 1983) are summarized in Appendix C. Both species have reported acute oral LD_{50}'s greater than 5,000 mg/kg body weight following intubation (oral dosage by tube to the crop) of up to 5,000 mg fosamine ammonium/kg with less than 50 percent mortality (actual value unreported) in ducks and no mortality in quail. In subacute oral studies, no mortalities were observed in ducks, and variable mortalities in quail, when fed concentrations of up to 10,000 ppm in feed for 5 days. Other than some evidence of reduced body weight gain and decreased food consumption in some studies, no abnormal effects attributable to fosamine ammonium were reported.

2.4 Mammals

Fosamine ammonium is a relatively new herbicide that has not been extensively tested. The potential health hazards from acute and subchronic exposure to fosamine ammonium have been evaluated in laboratory animals including rats, rabbits, guinea pigs, dogs, and sheep. The results of the toxicity tests summarized in Table 2-1, Appendix D, and Appendix E, are discussed below.

Based on data from acute and subchronic toxicity studies, fosamine ammonium is mildly toxic. Formulations cause mild eye, skin, and respiratory irritation. Fosamine ammonium is not a skin sensitizer. No teratogenic, reproductive, or embryo-fetal toxic effects have been induced with fosamine ammonium. Carcinogenicity studies have not been reported for fosamine. In five tests for mutagenicity, fosamine ammonium was nonmutagenic in bacterial and mammalian point mutation assays and an unscheduled DNA synthesis assay. It produced chromosome aberrations in an *in vitro* test, but produced no chromosome aberrations in an *in vivo* assay. Based on these results, fosamine ammonium does not appear to be mutagenic.

2.4.1 Acute Toxicity

Acute toxicity data are reported in Table 2-1 and Appendix D for oral, dermal, ocular, inhalation, and intraperitoneal exposure to formulated or unformulated types of fosamine ammonium.

2.4.1.1. Oral. In rats, acute oral LD_{50}'s greater than 5,000 mg/kg are reported for Krenite® (41.5 to 42.5% a.i.), and Krenite® S (41.1 to 41.5% a.i. plus surfactant) formulations (Table 2-1). Aqueous suspensions of the test materials were administered by intubation to rats of both sexes in single or repetitive doses. All animals were observed and/or treated for 14 days. Adverse toxic

effects induced by fosamine ammonium included weight loss, diarrhea, salivation, wet perinea, prostration, and irregular respiration. No gross pathologic abnormalities related to fosamine ammonium were observed.

Schneider and Kaplan (1983) reported two acute oral toxicity studies in female beagle dogs. In the first study, single intubation of a 42.5 percent a.i. formulation caused salivation and emesis minutes after dosing. The ALD* was greater than 12,800 mg/kg. In the second study, intubation of a 42 percent a.i. formulation yielded the same toxic effects and an LD_{50} greater than 15,000 mg/kg.

In guinea pigs, oral intubation of single doses of Krenite® caused no clinical signs at sublethal doses, and tremors, salivation, pallor, convulsions, and moribundity at lethal doses (Weed Science Society of America 1983 and Schneider and Kaplan 1983). The acute oral LD_{50} for fosamine ammonium is 7,380 mg/kg in this species.

2.4.1.2 Dermal. Fosamine ammonium is not a skin sensitizer but may induce mild transient skin irritation (Table 2-1). In rabbits, a 42 percent formulation (Krenite®) applied under occlusive cover to intact skin for 24 hours caused mild to moderate irritation that reversed to normal within 72 hours. A 41.1 percent plus surfactant formulation (Krenite®S) applied to intact and abraded rabbit skin in accordance with Federal Hazardous Substances Act (FHSA) procedures did not qualify as a primary skin irritant. Intact and abraded skin of guinea pigs were first treated with unformulated fosamine ammonium, then by 4 weekly intradermal injections of fosamine ammonium followed after 2 weeks by a topical challenge with fosamine ammonium. Transient mild erythema developed in some animals after the initial fosamine ammonium treatment; however, fosamine ammonium was not considered to be either a skin irritant or a skin sensitizer. When applied at dose of 1,683 mg a.i./kg body weight under occlusive bondage to intact rabbit skin for 24 hours followed by a 72-hour observation period, the 42 percent formulation (Krenite®) produced no mortalities and no clinical signs of toxicity. The 41.1 percent formulation plus surfactant (Krenite®S) applied 5,000 mg/kg under occlusive bondage to abraded skin of 5 male and 5 female rabbits followed by a 14-day observation period produced weight loss in both sexes and mild skin irritation and diarrhea in males. No clearly treatment-related gross pathological abnormalities were observed; the LD_{50} was greater than 5,000 mg/kg. Groups of 6 rabbits were treated with 0, 600, and 1,200 mg a.i./kg applied to intact skin under occlusive bondage for 6 hours a day over 10 consecutive days. Peripheral blood was collected prior to treatment and at sacrifice and all animals were subjected to gross and microscopic examination. No mortalities occurred and no evidence

*Acute lethal dose.

of compound-related abnormalities in clinical signs, growth, gross pathology, or histopathology were observed.

2.4.1.3 Eye Irritation. Krenite®, a 41.5 percent fosamine ammonium formulation without surfactant, produced no evidence of eye irritation in rabbits (Table 2-1). Krenite®S, a 41.5 percent fosamine ammonium formulation with surfactant, caused occassionally mild corneal opacity and iritis and moderate to severe conjunctival irritation in unwashed rabbit eyes with similar but milder effects in washed eyes. Five of 6 unwashed eyes were normal within 3 days and all eyes had returned to normal within 7 days of treatment (Appendix D).

2.4.1.4 Inhalation. Two inhalation studies summarized in Appendix D illustrate that fosamine ammonium in combination with a surfactant is more toxic to the respiratory tract than fosamine ammonium alone (Schneider and Kaplan 1983). Single whole body exposure of rats to Krenite®, 42 percent a.i., resulted in approximately lethal concentrations (ALC) for males and females of greater than 56 and 42 mg/l, respectively, based on calculations of material used and air flow. Rapid respiration and mild hyperemia but no change in weight gain were observed post exposure. Single head only exposure of rats to Krenite®S, a 41.1 percent fosamine ammonium plus surfactant formulation, for 4 hours resulted in LC_{50}'s of 3.30 and 2.75 mg/l in males and females, respectively, based on gravimetric analysis of exposure atmospheres. (Schneider and Kaplan 1983). Toxic symptoms which occassionally persisted for 14 days post exposure consisted of nasal and ocular discharge, corneal opacity, lung noise, weight loss, and weakness. Deaths resulted from lung, liver, and kidney congestion and associated circulatory-pulmonary arrest. Pathologic examinations revealed hemorrhagic thymus and brain, and corneal opacity.

2.4.1.5 Intraperitoneal Toxicity. Single intraperitoneal (ip) injections of a saline suspension of 42 percent a.i. formulation in rats followed by a 14-day observation period yielded an LD_{50} of 3,000 mg/kg (Appendix D). Tremors, prostration, irregular respiration, convulsions, and moribundity were observed at lethal doses in this study.

2.4.2 Subchronic Toxicity

No deaths and few adverse effects in response to subchronic oral administration of fosamine ammonium to rats, dogs, and sheep are noted in Table 2-1. Dietary provision of 0 to 5,000/10,000 ppm* of unformulated fosamine ammonium for 90 days resulted in no clinical,

*This nomenclature means animals were initially placed on the low concentration diets, but due to an absence of toxicity the dosage in the diet was increased to 10,000 ppm, the higher dose. The lengths of time on each diet were not specified for the rat study.

body weight, nutritional, hematological, biochemical, or gross pathological toxicity. Equivocal renal tubular effects were confirmed on subsequent histopathological examination to be unrelated to fosamine ammonium treatment (Schneider and Kaplan 1983). The NOEL was 5,000/10,000 ppm (Schneider and Kaplan 1983). In a second species, beagle dogs were provided 0 to 5,000/7,500/10,000 ppm* in their diet for 6 months. Increased stomach weights were observed in the high dose female dogs. No clinical, body weight, nutritional, hematalogical, biochemical, urinary, or gross pathological toxicity resulted (Schneider and Kaplan 1983). The NOEL for fosamine ammonium subchronic oral toxicity in dogs was 1,000 ppm. In a similar study, 0 to 2,000/2,500 ppm* of 41.5 percent a.i. formulation was fed to sheep for 90 days. No evidence of clinical, nutritional, body weight, motor reflex, or ophthalmic abnormalities were observed. The NOEL for this formulation in sheep was 2,000/2,500 ppm (Schneider and Kaplan 1983).

2.4.3 Chronic Toxicity

No data for two year or lifetime studies are available to date.

2.4.4 Reproductive and Teratogenic Effects

No reproductive, teratogenic, or embryo-fetal toxic effects have been induced with fosamine ammonium (Table 2-1). In a one generation, 2 litter, reproduction study, weanling rats were fed 0 to 5,000/ 10,000 ppm unformulated fosamine ammonium through a 90-day maturation period and through the subsequent production and weaning of 2 litters. No adverse effects on reproduction or lactation were observed. The NOEL for reproductive effects of fosamine ammonium was 5,000-10,000 ppm (Schneider and Kaplan 1983). In a teratogenicity study, pregnant rats were fed up to 10,000 ppm 42 percent a.i. formulation, Krenite®, from days 6 through 15 of gestation. No clinical, nutritional, or body weight abnormalities in maternal rats resulted. No evidence of teratogenicity or embryo-fetal toxicity was observed and the NOEL was 10,000 ppm (Schneider and Kaplan 1983).

2.4.5 Carcinogenicity

No data are available to date.

2.5 Mutagenicity

Appendix E summarizes five tests for evaluating the mutagenic potential of fosamine ammonium. All test were conducted with Krenite (41.5 or 42 percent Fosamine ammonium without surfactant).

*See footnote on previous page.

Four tests were negative. No mutagenicity was detected in the Salmonella typhimurium histidine revertant assay with or without microsomal activation. A point mutation assay for HGPRT deficient mutants in Chinese hamster ovary (CHO) cells was negative with and without activation. An in vitro bone marrow cytogenetic assay for detecting chromosomal aberrations was negative. In this assay, rats given up to 10,000 mg/kg fosamine formulation were sacrificed, and their bone marrow cells were subsequently examined. In the fourth assay, 41.5 percent a.i. fosamine formulation failed to induce unscheduled DNA synthesis in rat hepatocytes in vitro. The only test to give a positive response was an in vitro cytogenetic assay employing CHO cells. Chromosome damage was induced by a 41.5 percent a.i. fosamine ammonium formulation in the presence and absence of activation. On the basis of the above results, fosamine ammonium does not appear to be mutagenic because of its clastogenic activity in the cultured mammalian cells.

3.0 Environmental Fate

Fosamine ammonium is a relatively new herbicide used for post-emergence control of woody plants. Fosamine ammonium tends to be toxic to nontarget plant species, is rapidly adsorbed to soil particles, is rapidly degraded in soil, is relatively stable in water at neutral pH's, and has a very low toxicity to all species of animals in which it has been tested. It is absorbed by plant stems, buds, and foliage. Absorption through young stems appears to occur more readily than through foliage. Laboratory studies indicate that fosamine ammonium is translocated throughout the plant; however, in practice, effective action requires complete coverage of all parts of woody plants. Fosamine ammonium has a short persistence in plants and is metabolized to products which in turn have a relatively short persistence. It does not bioaccumulate in animals (Ghassemi et al. 1982; Norris et al. 1983; and Weed Science Society of America 1983).

3.1 Bioaccumulation and Metabolism

Very little information is available on the bioaccumulation and metabolism of fosamine ammonium in either plants or animals. Available data derived from Han (1971[b]), Chrzanowski (1983), Han and Krause (1979) and Chrzanowski et al. (1979) are reviewed in Ghassemi et al. (1981 and 1982), Norris et al. (1983), and Weed Science Society of America (1983).

3.1.1 Plants

Fosamine ammonium is usually applied to plants in the late summer and early fall and is absorbed by buds, stems and foliage. In most plants, effects of herbicide treatment are not evident until the following spring when buds fail to develop, or develop into miniature spindly leaves that do not provide adequate photosynthesis. Consequently, the plant dies. In some species of plants, such as pines and bindweed which are nondeciduous, leaves may turn brown immediately after application (Ghassemi et al. 1981 and Weed Science Society of America 1983).

In greenhouse studies, ^{14}C-labeled fosamine ammonium was applied to apple seedlings. When the material was applied to leaf surfaces, slower penetration was observed than when it was applied to the stems. The material applied to the leaf surface had a half-life of 2 to 3 weeks (Weed Science Society of America 1983 and Ghassemi et al. 1981 and 1982).

Metabolism of fosamine ammonium in plants was studied in the field in a pasture area in which small pin oak were surrounded by grass and clover (Chzranowski 1983). When applied at a rate of

12 lbs a.i./acre (13.4 kg/ha), ^{14}C-labeled fosamine ammonium had an average half-life of 7 days in the pasture flora. Two metabolites, carbamoylphosphonic acid and carboxyphosphonic acid, were detected in the flora and reached peak concentrations 2 weeks after application of the parent material and then rapidly declined. One year after treatment, no fosamine ammonium or the two metabolites were detected in the pasture at a detection limit < 0.05 ppm.

3.1.2 Animals

Fosamine ammonium does not bioaccumulate in animals and, once absorbed, is rapidly metabolized to carbamoylphosphonic acid, which with parent fosamine ammonium, is rapidly eliminated in feces and urine. Appendix F summarizes the available studies on the bioaccumulation of fosamine ammonium in rats, goats, and channel catfish.

Rats, preconditioned with 1,000 ppm fosamine ammonium in their diet, were administered a single dose of 57 to 58 mg radiolabeled fosamine ammonium per kg body weight by gavage (Chrzanowski et al. 1979 and Schneider and Kaplan 1983). Urine, feces, and expired air were collected and the animals were sacrificed 72 hours after ingesting the radiolabeled material. Approximately 87 percent of the total radioactivity was recovered in feces and up to 13 percent in urine. In one of the studies, only traces of radioactivity were found in expired air, the gut and the hide, and 13 percent of the total radioactivity in urine and feces was due to the metabolite, carbamoylphosphonic acid diammonium salt (Chrzanowski et al. 1979).

A lactating goat administered 50 ppm fosamine ammonium by capsule with the feed for 7 days eliminated 9 percent of the radioactivity in urine and 88.6 percent in feces. After 4 days feeding, peak concentrations of radioactivity in the goat milk reached only 0.008 ppm with less than 0.01 ppm of total residues excreted in milk. Total residues in all tissues and organs at sacrifice were less than 0.05 percent of the administered dose. Up to 30 percent of the total eliminated radioactivity represented the metabolite carbamoylphosphonic acid (Schneider and Kaplan 1983).

Han (1979b) exposed channel catfish for 4 weeks to water containing 1.1 ppm radiolabeled fosamine ammonium or to water over soil pretreated with radiolabeled fosamine ammonium at 15 ppm and aged 30 days prior to flooding. In both studies, the fish reached peak body tissue levels of fosamine ammonium after 2 to 3 weeks exposure. Maximum tissue concentrations were lower than those of the surrounding water. Concentrations of radioactivity in the fish tissue were reduced by 50 to 90 percent in both cases after 2 weeks depuration (exposure to water without the radiolabeled fosamine ammonium).

3.2 Soil

There are only limited data available on the behavior of fosamine ammonium in soil. These data, provided by Han (1979a), are summarized by Ghassemi et al. (1981 and 1982), by the Weed Science Society of America (1983), and by Norris et al. (1983). Greenhouse and field studies with radiolabeled compounds indicate that fosamine ammonium has a very short persistence with a half-life on the order of 1 week to 10 days in field and greenhouse studies, respectively. In the field, the metabolite carbomoylphosphonic acid (CPA) was found several days after initial soil treatment, but all [^{14}C] fosamine ammonium and [^{14}C] CPA had disappeared completely by 3 to 6 months after application. It is a very low mobility herbicide and is not readily leached from soil or carried away in precipitation runoff, despite its high water solubility, because it is readily and strongly adsorbed to soil particles. Because of this strong adsorption of fosamine ammonium to soil particles, there is little tendency for groundwater contamination or for surface waters to become contaminated without direct application of the material. Laboratory and field studies, though limited, indicate that photodecomposition and volatilization are not significant mechanisms for the loss of fosamine activity in soil. However, it is rapidly degraded by soil microorganisms. Laboratory biometer flask studies show microbial decomposition of [^{14}C] fosamine ammonium to $^{14}CO_2$ to be 45 to 75 percent complete after 90 days in the dark (Schneider 1984).

Field studies have been done with ^{14}C-labeled fosamine ammonium applied to surface soils at a rate of 10.1 lb/acre (11.3 kg/ha) (Han 1979a in Harvey 1983). When applied to silt loam in Delaware, very little movement of material was detected in the soil column and, after 6 months with over 36 inches (91.7 cm) of rainfall, more than half of the original applied radioactivity (58 percent) was located in the top 2 inches (5 cm) of soil. In fine sand in Florida, however, the radioactive material penetrated more rapidly despite less rainfall (40.4 cm), with only 6.4 percent of the original applied radioactivity in the top 2 inches (5 cm) of soil after 6 months. In this study, radioactivity lost from soil was assumed to be as $^{14}CO_2$ because both fosamine ammonium and its metabolite, carbamoylphosphonic acid, are essentially nonvolatile. Carbamoylphosphonic acid was detected in these soils as the fosamine ammonium metabolite but was lost from the Delaware soil within 6 months and from the Florida soil within 2 months. After 6 months in both soils, almost all of the residual radioactivity was identified as reincorporation products utilizing the radiolabeled carbon from the parent fosamine ammonium.

Fosamine ammonium is degraded relatively rapidly by soil microorganisms according to studies reported by Han (1979a in

Ghassemi et al. 1982 and in Weed Science Society of America 1983). Radiolabeled fosamine ammonium was incorporated into sandy loam and into silt loam to a concentration of 4 and 20 ppm each in laboratory flasks. Within 90 days, between 45 and 75 percent of the original applied radiolabeled carbon had been given off as $^{14}CO_2$ when the material had not been sterilized. Degradation in sterile flasks, however, was negligible for the first 20 to 30 days, and minimal $^{14}CO_2$ evolution observed subsequently was attributed to loss of sterility.

Han (1979a in Ghassemi et al. 1981 and 1982) also reported hydrolysis of fosamine ammonium to carbamoylphosphonic acid in soil. The environmental conditions of temperature, moisture, and pH that favor this reaction have not been reported, however.

3.3 Water

Fosamine ammonium is not readily degraded in water under neutral to mildly alkaline pH conditions in laboratory environments. The relative importance of various environmental factors studied on the rate of fosamine ammonium decomposition in water exclusive of microbial action appears to be pH, temperature, and uv light, in descending order. (Han 1979a in Ghassemi 1981). Under actual field conditions, microbial decomposition rates would be high (Schneider 1984). As discussed in Section 3.2, fosamine ammonium is strongly adsorbed onto soil particles and is not readily leached from soils into streams and ponds. Fosamine ammonium can enter aquatic systems as direct spray, as spray drift, or bound to soil runoff. Once in aquatic systems, it is likely that fosamine ammonium will adsorb onto bottom sediments or suspended sediments (Norris et al. 1983).

At 5 ppm concentration in the dark, aqueous solutions of fosamine ammonium are stable (with less than 3 percent decompositon) for 4 weeks at neutral pH (pH 7) and under slightly basic (pH 9) conditions. Under slightly acidic conditions (pH 5), however, it hydrolyzes rapidly (half-life of 10 days) to carbamoylphosphonic acid. At a higher concentration of 7,200 ppm, however, less than 3 percent decomposition occurred at all pH's observed (Han 1979a in Ghassemi et al. 1981 and 1982).

Data reported from photodecomposition studies with aqueous solutions of fosamine ammonium indicate that photodecomposition is very slow in the laboratory and should not play a significant role in loss of fosamine ammonium in natural aquatic systems. Han 1979a (in Ghassemi et al. 1981 and 1982) reported only 2 percent decomposition of fosamine ammonium in a 5 ppm solution exposed for 8 weeks to a light source equivalent to half of the sun's intensity at noon. A 5 ppm solution at pH 5, exposed to sunlight for 4 weeks in Wilmington, Delaware, was only slightly degraded.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to fosamine ammonium during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved fosamine ammonium. Calculations of exposure to fosamine ammonium in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to fosamine ammonium are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Fosamine Ammonium Application

Available application data for fosamine ammonium use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to fosamine ammonium differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of fosamine ammonium in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Fosamine ammonium is readily adsorbed to soil particles. It persists 7 to 10 days in soil and is rapidly degraded by microorganisms. Consequently, the potential for incidental exposure of humans and wildlife to fosamine ammonium is low.

4.2 Exposure

Estimates of potential exposure to fosamine ammonium are based on aerial as well as ground application. The maximum aerial application rate for fosamine ammonium (see Table 1-1) is 10.5 lb a.i./acre, while the compound is applied from the ground (ground foliar) at rates as high as 8.0 lb a.i./acre. In both aerial application and ground

Table 4-1

USDA Forest Service Application Data for Fosamine Ammonium (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Krenite (10)	Ground Foliar	6.1	61	5.1	11.4	6.3	20	Engineering (5) Wildlife (3) Range (1) Noxious Weeds (1)

Source: Gross 1983

foliar application, fosamine ammonium is applied as a liquid. The calculations in Table 4-2 that are based on aerial application (e.g. spray/observer dose, wildlife dermal and oral exposures, and water concentrations) assume an application rate of 10.5 lb a.i./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose) assume an application rate of 8.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of fosamine ammonium and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of fosamine ammonium to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for fosamine ammonium are based on exposures on a per pound per acre application rate multiplied by 10.5 lb/acre for observers and 8.0 lb/acre for backpack sprayers. In the case of the aerial spray observer, dose estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. This absorption rate is based on the high water solubility and low solubility in organic solvents.

At 10.5 lb a.i./acre aerial application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.42 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 8.0 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.32 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Fosamine Ammonium

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg[b]		
Observer	up to 0.42 mg/kg		
Backpack Sprayer	0.32 mg/kg		
Environmental Exposure			
Fish	0.11 ppm (Immersion)[c]		
Rabbits	4.23 mg/kg	Negligible	11.0 mg/kg
Deer	1.06 mg/kg	Negligible	15.1 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.002 mg/kg (2 days)
(Rabbits)	NA	NA	0.053 mg/kg (2 days)
(Deer)	NA	NA	0.063 mg/kg (2 days)
(Water)	NA	NA	0.0042 mg/kg (1 day)
(Berries)	NA	NA	0.0105 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0053 mg/kg (1 day)
Toxicity Summary	Acute LD_{50} >1,683 mg/kg in rats and rabbits	LC_{50} >2.75 ppm in rats	Acute oral LD_{50}'s >5,000 mg/kg in rats, Beagle dogs, and guinea pigs.
	Most studies, LC_{50} >1,000 ppm for fish		

[a]Assumes aerial application at 10.5 lb active ingredient per acre and ground foliar application at 8.0 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure.

[c]Fish exposure results from immersion in water containing fosamine ammonium.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by fosamine ammonium application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 10.5 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 4.2315 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 1.0605 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus, a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of fosamine ammonium vapor would be negligible since the vapor pressure of fosamine ammonium is low. Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds

adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of fosamine ammonium in water, calculated as:

Water concentration: 0.105 ppm (0.01 ppm at 1 lb/acre aerial application rate)

This concentration of fosamine ammonium represents the estimated maximum concentration immediately after spraying. If heavy rainfall occurred immediately after spraying, higher transient concentrations could occur in streams and ponds. Han (1979a in Ghassemi et al. 1981 and 1982) reported very slow decomposition of fosamine ammonium in the laboratory. Depending on the pH of the water and amount of light, the decomposition varied between 4 and 8 weeks. However, in natural environments, microbial decomposition of fosamine ammonium would be high.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of fosamine ammonium by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed aerially at 10.5 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 15.12 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 11.025 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of fosamine ammonium in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.105 ppm

- bioconcentration: 1.0 (actual bioconcentration for fosamine ammonium is <1.0 in most studies) (Han 1979b)

Fish tissue concentration = 0.105 ppm (0.105 mg/kg)

Deer and rabbit tissue concentrations include fosamine ammonium accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied fosamine

ammonium is absorbed at a rate of 10 percent as discussed in Section 4.2.1 with regard to spray observer absorption rates. It is also assumed that oral exposure of deer and rabbit to fosamine ammonium lasts only for 2 days due to the relatively short environmental persistence of fosamine ammonium and that only 10 percent of the ingested fosamine ammonium is assimilated. This latter assumption is quite conservative since studies summarized in Appendix F indicate that much less than 10 percent of administered fosamine ammonium is found in tissues. Deer tissue concentrations of fosamine ammonium are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 1.0605 mg/kg (dermal exposure) x 0.1 = 0.10605 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 15.120 mg/kg/day (oral exposure) x 0.1 x 2 days = 3.024 mg/kg.

Total Deer Tissue concentration = 0.10605 mg/kg + 3.024 mg/kg = 3.1300 mg/kg (3.130 ppm).

Rabbit tissue concentrations of fosamine ammonium are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 4.2315 mg/kg (dermal exposure) x 0.1 = 0.42315 mg/kg.

Oral Exposure contribution assumes 10 percent absorption of two-days feeding, or 11.025 mg/kg/day (oral exposure) x 0.1 x 2 days = 2.2050 mg/kg.

Total Rabbit Tissue Concentration = 0.42315 mg/kg + 2.205 mg/kg = 2.6281 mg/kg (2.628 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to fosamine ammonium from eating meat are derived:

Fish meat: 0.105 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0021 mg/kg

Deer meat: 3.13 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0626 mg/kg

Rabbit meat: 2.628 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0526 mg/kg

Water and plant consumption by man would result in the following oral exposures to fosamine ammonium based on assumptions stated in the General Introduction to the Herbicide Background Statements at an application rate of 10.5 lb a.i./acre (aerial):

Water:	0.0042 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries:	0.0105 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms:	0.0053 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of fosamine ammonium to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environment exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rats and rabbits, rat inhalation results, rat, beagle dog, and guinea pig oral LD_{50}'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels for USDA Forest Service applications.. Based on acute feeding studies in three species, the LD_{50}'s are substantially greater than 5000 mg/kg. Assuming a 100-fold margin of safety for extrapolation to man (a factor of 10 for inter-species variability and a factor of 10 for human intra-species variability), one can assume that 5000 mg/kg divided by 100, or a 50 mg/kg oral dose in man, will not result in adverse acute effects. The estimated human exposures from ingestion of water, game, and plant material are well below 50 mg/kg per day.

Small mammal studies have shown that fosamine ammonium without surfactant when applied to the eye is not an irritant; however, fosamine ammonium with surfactant is an irritant to the iris and conjunctiva. Fosamine ammonium fed at rates up to 10,000 ppm in feed does not result in birth defects (in rats). Fosamine ammonium fed at rates 5,000/10,000 ppm did not impair reproduction or lactation performance in rats. Fosamine ammonium was not mutagenic in four of five systems tested. No carcinogenicity data are available. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive, mutagenic, or chronic toxic effects to individuals from fosamine ammonium use, if proper care is taken during application.

Appendix A

Summary of Fosamine Ammonium Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Fosamine ammonium salt (42% formulation)	Bees (Insecta)	Contact (dermal adsorption)	48 hours	LC_{50} >10,000 ppm. Bees sprayed with test material at concentrations of 0, 25, 50, 100, 250, 500, 1,000, and 10,000 ppm.	Sporadic mortality observed at concentrations of 100 ppm and higher.	Schneider and Kaplan 1983
Fosamine ammonium salt (unformulated)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	LC_{50} = 1,524 ppm (95% confidence limits of 1,310 to 1,720 ppm)	Animals not fed during experiment, 24-hour old individuals used.	Schneider and Kaplan 1983
Fosamine ammonium	Soil fungi: Aspergillus niger, A. terreus, Penicillium citrinum, Gibberella saubinetti, Fusarium sp., Alternaria sp., Rhizoctonia solani, and Pythium sp.	Agar plate bioassay	--[a]	Little or no fungitoxicity observed at treatment rates up to 100 ppm.	--	Dupont data in Ghassemi et al. 1981 and 1982 and Han Krause 1979
Fosamine ammonium	Soil bacteria and fungi in 3 agricultural soils (fine sand from Florida, sandy loam from Delaware, and silt loam from Illinois)	Laboratory	8 weeks	Populations and species of bacteria and fungi unaltered when treated with 10 ppm fosamine ammonium.	--	DuPont data in Ghassemi et al. 1981 and 1982 and Han Krause 1979

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Fosamine ammonium	Soil-nitrifying bacteria in 2 agricultural soils (sandy loam and silt loam from Delaware)	Laboratory	5 weeks	No effect on populations or activities soil nitrifying bacteria when treated with 0.5, 5, and 20 ppm fosamine ammonium.	--	Han 1979a, Han and Krause 1979, both in Ghassemi et al. 1981 and 1982
Krenite®	Algae (*Nostoc sp.*) isolated from lichen (*Pettigera praetextata*)	Laboratory	8 hours	Drastic reduction in rate of nitrogen fixation when treated with 100 ppm fosamine ammonium with total inhibition after 8 hours.	--	Hallborn and Bergman 1979 in Ghassemi et al. 1981 and 1982

[a]Data are not available or note applicable.

Appendix B

Summary of Fosamine Ammonium Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Fosamine ammonium salt (42% formulation)	Rainbow trout	Surface absorption, static bioassay	24 hours 96 hours	LC_{50} >1,000 ppm LC_{50} >1,000 ppm	Fish exposed to 0, 100, 240, 490, 750, and 1,000 ppm. Fish not fed 24 hours before, or during, test. No effects observed at 1,000 ppm.	Schneider and Kaplan 1983
Fosamine ammonium salt (42% formulation)	Bluegill sunfish	Surface absorption, static bioassay	24 hours 96 hours	LC_{50} >1,000 ppm LC_{50} = 670 ppm (95% confidence limits are 378 to 1,190 ppm).	Fish exposed to 0, 100, 240, 490, 750, and 1,000 ppm. Fish not fed 24 hours before, or during, test. No effects observed at 240 ppm.	Schneider and Kaplan 1983
Fosamine ammonium salt (42% formulation)	Fathead minnow	Surface absorption, static bioassay	24 hours 96 hours	LC_{50} >1,000 ppm LC_{50} >1,000 ppm	Fish exposed to 0, 100, 240, 490, 750, and 1,000 ppm. Fish not fed 24 hours before, or during, test. No effects observed at 1,000 ppm.	Schneider and Kaplan 1983
Fosamine ammonium salt (42% formulation)	Coho salmon	Surface absorption, both static bioassay and bioassay with 24-hour test solution renewal.	96 hours	LC_{50} = 8,290 ppm (without renewal) LC_{50} = 9,812 ppm (with renewal)	Avoidance EC_{50} = 295 ppm with a threshold of 8.9 ppm (no strong preferences observed). Acute stress (leucocrit values) EC_{50} = 198 ppm with a threshold of 4 ppm.	Schneider and Kaplan 1983

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Krenite	Coho salmon Alevin (yolk-sac fry)	Surface absorption (partial life-cycle study)	96 hours	LC_{50} = 618 ppm (including post-exposure mortality)	Alevin was the most sensitive stage. Post-exposure mortalities occurred after a 96-hour exposure in all life stages. Values for 96-hour LC_{50} for swim-up fry varied 12-fold when diluent waters varied in pH, hardness and alkalinity. No groups surviving previous exposure showed any latent effects throughout observation period in freshwater. Toxicity for 12-day continuous exposure was greater than for 4-day exposure and was dependent upon specific life stage. Threshold concentrations are 618 ppm (4-day) and 250 ppm (12-day).	McLeay and Gordon 1980 in Norris et al. 1983 and Schneider and Kaplan 1983
	Egg Stages		96 hours	LC_{50}'s from 25,377 ppm to 64,450 ppm (including post-exposure mortality)		
	Yearling pre-smolts		96 hours	LC_{50} = 7,014 ppm (LC_{50} = 3,295 ppm including post-exposure mortality)		
	Finglerlings		96 hours	LC_{50} = 5,361 ppm (LC_{50} = 2,669 ppm including post-exposure mortality)		

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Krenite®	Rainbow trout Alevin (yolk-sac fry) Egg stages	Surface absorption (partial life-cycle study)	96 hours	LC_{50} = 367 ppm (including post-exposure mortality) LC_{50}'s from 1,456 ppm to 64,450 ppm (including post-exposure mortality)	Alevin was the most sensitive stage. Post-exposure metalities occurred after a 96-hour exposure in all life stages. Values for 96-hour LC_{50} for swim-up fry varied 14-fold when diluent waters varied in pH, hardness, and alkalinity. No groups surviving previous exposure showed any latent effects throughout observation period in fresh water. Toxicity for 12-day continuous exposure was greater than for 4-day exposure, and was dependent upon specific life-stage. Threshold concentration is 367 ppm (4-day exposure).	McLeay and Gordon 1980 in Norris et al. 1983 and in Schneider and Kaplan 1983
Krenite®	Coho salmon (yearlings)	Surface absorption, static bioassay	6 days in freshwater and Krenite®	Fish exposed to 0, 0.25, 0.50, 1.0 10, 50, 100, 200 ppm. No mortality observed.	Only minimal mortality occurred when exposed fish transferred to seawater. No apparent effect of Krenite® on gill activity (ATP-ase).	Lorz et al. 1979

Appendix C

Summary of Fosamine Ammonium Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Fosamine ammonium salt (unformulated)	Mallard duck (male and female)	Acute oral (intubation into the crop)	--[a]	LD_{50} >5,000 mg/kg body weight. Following overnight fast, ducks received single doses of 0, 312.5, 625, 1,250, 2,500, or 5,000 mg/kg body weight and observed for 14 days.	Surviving ducks exhibited no clinical abnormalities, males with decreased body weight gain at all treatment doses.	Schneider and Kaplan 1983
Fosamine ammonium salt (unformulated)	Bobwhite quail (male and female)	Acute oral (intubation into the crop)	--	LD_{50} >5,000 mg/kg body weight. Following overnight fast, quail received single doses of 0, 312.5, 625, 1,250, 2,500, or 5,000 mg/kg body weight and observed for 14 days.	No deaths occurred. Males exhibited decreased body weight gain with herbicide treatment.	Schneider and Kaplan 1983
Fosamine ammonium salt (unformulated)	Mallard duck	Subacute oral	Birds fed compound for first 5 days of an 8-day study.	LC_{50} >10,000 ppm. Birds fed 0, 625, 1,250, 2,500, 5,000 or 10,000 ppm in feed.	Birds 14 days old at start of study. No mortalities observed, food consumption depressed in treated birds but body weight gain through study was normal.	Schneider and Kaplan 1983
Fosamine ammonium salt (unformulated)	Bobwhite quail	Subacute oral	Birds fed compound for first 5 days of an 8-day study.	LC_{50} >10,000 ppm. Birds fed 0, 625, 1,250, 2,500, 5,000 or 10,000 ppm in feed.	Birds 14 days old at start of study. Variable mortality observed, mean food consumption and body weight gain were slightly less than controls.	Schneider and Kaplan 1983

[a]Data are not available or not applicable.

Appendix D

Summary of Fosamine Toxicity in Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Fosamine ammonium salt (41.5% a.i., Krenite® Brush Control Agent)	Rat (male, Crl:CD	Single dose of an aqueous suspension administered by intubation to groups of 5 animals per dose. Animals weighed and observed for 14 days.	LD_{50} = 24,400 mg/kg body weight with 95% confidence limits of 18,000 and 33,000 mg/kg body weight. Observed weight loss, diarrhea, irregular respiration, and belly-to-cage posture.	--[a]	Weed Science Society of America 1983; Toxic Effects Registry 1980 and Schneider and Kaplan 1983
Fosamine ammonium salt (41.5% a.i. + surfactant, Krenite®S Brush Control Agent)	Rat (male and female, Crl:CD	Single dose of an aqueous suspension administered by intubation to 1 group of 5 male rats and 1 group of 10 female rats at 5,000 mg/kg body weight. Animals weighed and observed for 14 days. All males and 5 females subjected to gross pathological examination at necropsy.	LD_{50} >5,000 mg/kg body weight. Sporadic weight loss in both sexes; stained and wet perinea and diarrhea in 1 male. Observed no compound-related gross pathological changes.	--	Weed Science Society of America 1983 and Schneider and Kaplan 1983.
Fosamine ammonium salt (42% a.i., Krenite® Brush Control Agent)	Guinea pigs, (male)	Single dose of a 90% aqueous suspension administered by intubation to groups of 5 animals per dose. Animals observed for 14 days.	LD_{50} = 7,380 mg/kg body weight with 95% confidence limits of 10,170 and 5,360 mg/kg body weight. Observed tremors, salivation, pallor, convulsions, and moribundity at lethal doses. No clinical signs at sublethel doses.	--	Weed Science Society of America 1983 and Schneider and Kaplan 1983
Fosamine ammonium salt (41.5% a.i. + surfactant, Krenite®S)	Rat (male, Crl:CD	Single dose of an aqueous suspension administered by intubation to groups of 10 animals per dose. Animals weighed and observed for 14 days. Animals from each dose were subjected to gross pathological examination at necropsy.	LD_{50} = 7,295 mg/kg body weight with 95% confidence limits of 6,767 and 7,581 mg/kg body weight. Observed diarrhea and rapid respiration for 1 to 2 days after dosing, stained perinea from 2 to 4 days after dosing, tremors several days after dosing, and belly-to-cage posture, wet perinea, and prostration on day of dosing. Observed no compound-related gross pathological changes.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Concluded)		
Fosamine ammonium salt (42.5% a.i., Krenite®)	Dog (female, beagle)	Single dose of 15,000 mg/kg body weight administered by intubation to 1 dog followed by a 14-day observation period.	ALD >12,800 mg/kg body weight. Observed slight salivation and vomiting within 10 minutes of dosing.	--	Schneider and Kaplan 1983
Fosamine ammonium salt (42% a.i., Krenite®)	Dog (female, beagle)	Single dose of 15,000 mg/kg body weight (128 m) administered by intubation to 4 dogs followed by a 14-day observation period.	LD_{50} >15,000 mg/kg body weight. Observed slight salivation and vomiting within 15 minutes of dosing.	--	Schneider and Kaplan 1983
Fosamine ammonium salt (42% a.i., Krenite®)	Rat (male, Crl:CD)	Administration of a 20% aqueous suspension at a dose of 2,200 mg/kg/day by intubation 5 times a week for 2 weeks to a group of 6 rats. At 4 hours and 14 days after the last dose 3 test and control animals sacrified for gross and microscopic pathological examination.	No deaths. Observed mild diarrhea during the dosing period. No histologic evidence of gastrointestinal irritation. Observed no compound-related gross or microscopic pathologic abnormalities.	--	Schneider and Kaplan 1983
			Acute Demal Toxicity		
Fosamine ammonium salt (41.1% a.i. + surfactant, Krenite®)	Rabbit (male and female)	Treated 5 rabbits per sex with single dose of 5,000 mg/kg body weight. Material applied to abraded skin and covered with gauze and occlusive bandage for 24 hours. Observed animals for 14 days then sacrified. Two animals of each sex subjected to gross pathological examination of treated skin.	LD_{50} >5,000 mg/kg body weight. Observed weight loss in both sexes, mild skin irritation and diarrhea in males. No clearly compound-related gross pathological abnormalities apparent.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Continued)		
Fosamine ammonium salt (42% a.i., Krenite®)	Rabbit (male)	Treated groups of 6 rabbits with 10 ml of 1,683 mg a.i./kg body weight. Applied to intact skin and covered by gauze and occlusive bandage for 24 hours followed by a 72-hour observation period.	LD_{50} >1,683 mg a.i./kg body weight. Observed no mortalities and no clinical signs of toxicity.	--	Weed Science Society of America 1983 and Schneider and Kaplan 1983
Fosamine ammonium salt, (42% a.i., Krenite®)	Rabbit (male)	Treated 6 rabbits with 0, 600, and 1,200 mg a.i./kg body weight. Material applied to intact skin and covered with gauze and occlusive bandage daily for 6 hours/day for 10 days. Peripheral blood collected 5 days prior to treatment and at sacrifice for clinical evaluation. All animals sacrificed 1 day after the tenth exposure and subjected to gross and microscopic evaluation.	No deaths. Observed no evidence of compound-related growth or clinical-, gross-, or histopathological abnormalities.	--	Schneider and Kaplan 1983
Fosamine ammonium salt, (42% a.i., Krenite®)	Rabbit (male)	Treated 6 rabbits with 10 ml of 1,683 mg a.i/kg body weight. Material applied to intact skin and covered by gauze and occlusive bandage for 24 hours followed by a 72-hour observation period.	Observed mild to moderate skin irritation at 24 hours and no irritation at 72 hours.	--	Schneider and Kaplan 1983
Fosamine ammonium salt, (41.1% a.i. + surfactant, Krenite® S)	Rabbit (male)	Two intact and 2 abraded skin areas on each of 6 rabbits treated with 0.5 ml of test material then covered for 24 hours. Treatment sites scored according to Draize after removal of patches at 24 and 72 hours.	Not a primary skin irritant. Draize scores ranged from 0.75 to 2.75 (>5.0 required for primary irritant classification).	--	Schneider and Kaplan 1983

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Concluded)		
Fosamine ammonium salt (unformulated)	Guinea pigs (male)	Applied 0.05 ml of 25 or 50% aqueous suspensions to intact or abraded skin of 10 guinea pigs. Induction for sensitization by 4 weekly sacral intradermal injections of 1% aqueous (0.1 ml) suspensions in 5 animals. Nine topical applications of 50% aqueous suspensions over the same 4-week period in the other 5 animals. Thirteen days after the last induction dose, the animals were topically challenged with 50% aqueous suspensions on intact and abraded skin.	Observed transient mild erythema in some animals after initial treatment with 50% suspension. No sensitization. Material not considered to be an irritant or sensitizer.	--	Weed Science Society of America 1983
			Eye Irritation		
Fosamine ammonium (41.5% a.i. Krenite® Brush Control Agent)	Rabbit	0.1 ml solution applied to eye	No evidence of eye irritation.		Weed Science Society of America 1983
Fosamine ammonium salt (42% a.i., Krenite®)	Rabbit	Treated right eyes of 6 rabbits with 0.1 ml of test material. Examined eyes with hand-slit lamp at 24, 48, and 72 hours after treatment.	No ocular effects. Not an irritant.	--	Schneider and Kaplan 1983
Fosamine ammonium salt (41.1% + surfactant, Krenite® S)	Rabbit (male)	Treated right eyes of 9 rabbits with 0.1 ml of test material. After 20 seconds, washed 3 eyes. The remaining 6 eyes left unwashed. All eyes examined with a hand-slit lamp at 1 to 7 days and scored according to Draize.	Mild to no corneal opacity and severe to moderate conjunctival irritation in unwashed eyes. One unwashed eye showed slight iritic irritation. Five of 6 unwashed eyes normal within 3 days, the last unwashed eye normal within 7 days. Similar but milder effects in washed eyes. Two of 3 washed eyes normal within 3 days, the remaining washed eye normal within 7 days.		Weed Science Society of America 1983; Schneider and Kaplan 1983

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Inhalation Toxicity		
Fosamine ammonium salt (42% a.i., Krenite®)	Rat (male and female, Crl:CD)	Whole body exposure of groups of 6 rats per sex for single, 1-hour periods to atmospheres of test material in 16-liter chambers. Concentrations calculated from material used and airflow.	One hour approximate lethal concentration (ALC) male >56.6 mg/1; acute female >42.0 mg/1; no mortalities. All animals exhibited face pawing, grooming, rapid respiration, mild hyperemia, and wet fur during exposures. Normal weight gain.	--	Weed Science Society of America 1983 and Schneider and Kaplan 1983
Fosamine ammonium salt (41.1% a.i. + surfactant, Krenite®S)	Rat (male and female, Crl:CD)	Head only exposure of groups of 10 rats per sex for single, 4-hour periods to atmospheres of test material in 50 glass chambers. Rats weighed and observed daily for 14 days post-exposure. All mortalities and up to 3 survivors per sex per group subjected to gross and histopathologic examination. Chamber atmospheres analyzed gravimetically at half hour intervals. Aerodynamic particle size determined by cascade impactor.	Males: LC_{50} = 3.30 mg/1 (dry weight) with 95% confidence limits of 2.57 and 4.48 mg/1. Females: LC_{50} = 2.75 mg/1 (dry weight) with 95% confidence limits of 1.93 and 3.36 mg/1. Observed nasal and ocular discharge, corneal opacity, lung noise, weaknss, moderate to severe weight loss, alopecia, and coldness to touch. Toxic symptoms occasionally persisted through 14 day post-exposure period. Mortalities resulted from lung, liver, and kidney congestion and associated circulatory-pulmonary arrest. Histopathology revealed hemorrhagic thymus and brain. Corneal opacity was the only persistent pathologic abnormality among survivors at 14 days.	--	Schneider and Kaplan 1983
			Intraperitoneal Toxicity		
Fosamine ammonium salt (42% a.i., Krenite®)	Rat (male Crl:CD)	Administered single dose by intraperitoneal injection of a 90% suspension in 0.9% saline to groups of 5 animals per dose. Survivors observed, for 14 days.	LD_{50} = 3,000 mg/kg body weight. Observed belly-to-cage posture, tremors, and prostration at sublethal and lethal doses. Irregular respiration, pallor, convulsions, and moribundity observed at lethal doses.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity		
Fosamine ammonium salt (unformulated)	Rat (male and female, Crl:CD)	Dietary concentrations of 0, 200, 1,000, and 5,000/10,000 ppm administered to groups of 10 male and 10 female weanling rats for 90 days. Clinical pathological evaluations conducted at 30, 60, and 90 days.	No deaths. Observed no clinical, nutritional, hematological, biochemical, or gross patho-logical evidence of compound-related toxicity No body weight or urinary abnormalities. Equivocal renal tubular effects at high dose confirmed on subsequent analysis to be unrelated to test material. The NOEL - 5,000/10,000 ppm.	--	Schneider and Kaplan 1983
Fosamine, (unspecified)	Rat (male)	Ten doses of 2,200 ppm per day over a 2-week period.	No histopathologic evidence of cumulative toxicity; no adverse effects except mild diarrhea.	--	Weed Science Society of America 1983 and Ghassemi et al. 1981
Fosamine ammonium salt (unformulated)	Dog (male and female, beagle)	Fed 0, 200 1,000, or 5,000/7,500/10,000 ppm for 6 months to groups of 4 male and 4 female 14 to 16 month old beagle dogs. High dose dogs received 5,000 ppm for 1 week, 7,500 ppm for 2 weeks and 10,000 ppm for the remainder of the study. Clinical pathological examinations conducted twice before dosing initiated and following 1 to 6 months of treatment. No histopathology carried out.	No deaths. Increased relative stomach weights in high dose female dogs. Observed no clinical, body weight, nutritional, hematological, biochemical, urinary, gross pathological evidence of a compound-related toxic effect. The NOEL = 1,000 ppm.	--	Schneider and Kaplan 1983
Fosamine ammonium salt (41.5% a.i., Krenite®)	Sheep (male and female)	Fed 0, 1,000, and 2,000/2,500 ppm to groups of 3 male and 3 female 8 to 10 month-old sheep for 90 days. Motor reflex and ophthalmic examinations conducted weekly.	No deaths. No evidence of clinical, nutritional, body weight, motor reflex, or ophthalmic abnormalities. The NOEL = 2,000/2,500 ppm.	--	Schneider and Kaplan 1983

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity No 2-year or lifetime data available		
			Reproduction and Teratogenicity		
Fosamine ammonium salt (unformulated)	Rat (Crl:CD)	1 generation/2 litter oral reproduction study. Fed 0, 200, 1,000, and 5,000/10,000 ppm to groups of 6 male and 6 female weanling rats through a 90-day maturation period and through the production and weaning of 2 litters.	No adverse effects on reproduction or lactation. The NOEL for reproductive effects was 5,000/10,000 ppm.	--	Schneider and Kaplan 1983
Fosmaine ammonium salt (42% a.i., Krenite®)	Rat (female, Crl:CD)	Fed 0, 200, 1,000, and 10,000 ppm to pregnant rats from day 6 through day 15 of gestation. Rats sacrificed on day 21 of gestation. Maternal and fetal examinations carried out.	No clinical, or nutritional signs of toxicity in maternal animals. No body weight abnormalities. No evidence of embryo-fetal toxicity or teratogenicity observed at any test concentration. The NOEL for embryo-fetal toxicity and teratogenicity was 10,000 ppm.	--	Schneider and Kaplan 1983
			Carcinogenicity No data available		

[a]Data are not available or not applicable.

Appendix E

Summary of Fosamine Ammonium Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Fosamine ammonium salt (42% a.i., Krenite®)	Salmonella typhimurium (5 strains, unspecified)	Salmonella/Microsome Assay: Plate incorporation assay with incubation in the presence and absence of activation system. Revertant colonies then counted.	Nonmutagenic	--[a]	Schneider and Kaplan 1983
Fosamine ammonium salt (41.5% a.i., Krenite®)	Chinese Hamster Ovary (CHO) cells	Point Mutation Assay: Incubated CHO cells in treatment medium in the presence and absence of activation system, subcultured to allow growth and expression of cytotoxicity, then seeded in medium with and without selective agents to allow enumeration of HGPRT deficient mutants.	Nonmutagenic	--	Schneider and Kaplan 1983
Fosamine ammonium salt (41.5% a.i., Krenite®)	Chinese Hamster Ovary (CHO) cells	In Vitro Cytogenetic Assay: Exposed CHO cells to test material in the presence and absence of activation system, then treated cells with colcemid to arrest in metaphase. Subsequently, cells harvested and chromosome preparations made and scored.	Chromosome damage produced under nonactivated conditions at 1.6 and 3.2% test material by volume and under activated conditions at 1.4, 2.8, and 5.7% test material by volume.	--	Schneider and Kaplan 1983
Fosamine ammonium salt (42% a.i., Krenite®)	Rat (Sprague-Dawley-CD)	In Vivo Bone Marrow Cytogenetic Assay: Administered single doses of 0, 1,000, 3,000, and 10,000 mg/kg body weight by intubation to groups of 12 male and 12 female rats. Three rats per sex per group administered colchicine at 4, 10, 22, and 46 hours after treatment to inhibit mitosis and arrest cells in metaphase. Subsequently the rats were sacrificed and bone marrow cells collected from femurs of each rat were prepared and examined.	No compound-related chromosomal aberrations detected. No mortalities. Two intermediate and 3 high dose animals noted to have soft feces at first post-treatment observation. No other clinical or consistent body weight changes seen.	--	Schneider and Kaplan 1983
Fosamine ammonium salt (41.5% a.i., Krenite®)	Rat hepatocytes in vitro	Unscheduled DNA Synthesis Assay: Treated freshly isolated rat hepatocytes with test material followed by radiolabeled thymidine. Incorporation of label into DNA was quantitated by autoradiography.	No induction of unscheduled synthesis observed	--	Schneider and Kaplan 1983

[a] Data are note available or not applicable.

Appendix F

Summary of Metabolism Studies with Fosamine Ammonium

Formulation	Organism	Dose	Comments	References
Fosamine ammonium salt (unformulated, ^{14}C-labeled)	Rat (male, Charles River) (single animal)	Single 58 mg/kg dose following 18 days of preconditioning with 1,000 ppm in feed.	Urine, feces and expired air collected. Rat sacrificed at 72 hours and tissues sampled. Radioactivity eliminated within 72 hours, approximately 88% of total dose in feces, 11% in urine, and none in expired air. Approximately 33% of activity in urine and 32% in feces was intact fosamine ammonium. Metabolites were not identified.	Schneider and Kaplan 1983
Fosamine ammonium salt (unformulated, ^{14}C-labeled)	Rat (male, Charles River) (2 animals)	Single 57 mg/kg dose following 18 days of preconditioning with 1,000 ppm in feed.	Urine, feces and expired air collected. Rats sacrificed at 72 hours and tissues sampled. Radioactivity eliminated within 72 hours, approximately 87% of total dose in feces, 13% in urine, and traces (0.1 to 0.2%) in gut, hide, and expired air. No radioactivity (0.05%) detected in body tissues. In urine and feces, 87% of activity was intact fosamine ammonium, 13% was the metabolite carbamoylphosphonic acid diammonium salt.	Chrzanowski et al. 1979
Fosamine ammonium salt (unformulated, ^{14}C-labeled)	Goat (lactating)	Seven daily doses at 50 ppm by capsule with feed.	Milk, urine and feces collected daily, expired air collect only on 7th day, and goat sacrificed 24 hours after last dose and tissues sampled. Approximately 9% of total radioactivity in urine, 88.6% in feces, 0.22% of dose in expired air, and 0.05% in tissues. Radioactivity in milk reached peak of 0.008 ppm on day 4. In urine and feces, 70% of activity was fosamine ammonium, 20 to 30% was intact the metabolite carbamoylphosphonic acid.	Schneider and Kaplan 1983

Appendix F (Concluded)

Formulation	Organism	Dose	Comments	References
Fosamine ammonium salt (unformulated, ^{14}C-labeled)	Channel catfish (50 fish)	Exposed to 1.1 ppm concentration under static, unaerated conditions for 4 weeks followd by 2 weeks in water without herbicide.	Fish and water sampled on days 3, 7, 14, 21 and 28 and fish sampled after 3, 7, and 14 days in water without herbicide. Radioactivity in fish plateaued after 2 to 3 weeks in water with herbicide with a bioaccumulation factor less than 1. After 2 weeks in water without herbicide, fish tissue levels of radioactivity reduced 50 to 90%.	Han 1979b
Fosamine ammonium salt (unformulated, ^{14}C-labeled)	Channel catfish (50 fish)	Exposed to water over soil pretreated to contain 15 ppm fosamine ammonium and aged 30 days prior to flooding. Fish kept under static, unaerated conditions for 4 weeks followed by 2 weeks in water without herbicide.	Fish and water sampled on days 3, 7, 14, 21 and 28 and fish sampled after 3, 7, and 14 days in water without herbicide. Radioactivity in fish plateaued after 2 to 3 weeks in water with herbicide with a bioaccumulation factor less than 1. After 2 weeks in water without herbicide, fish tissue levels of radioactivity reduced 50 to 90%.	Han 1979b

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Herbicide Background Statement: Glyphosate

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Glyphosate Summary

Major Trade Names: Roundup®, Rodeo®

Chemical Name: N-(phosphonomethyl)glycine

Major Forestry Applications: Applied as a postemergence spray for the control of weeds and brush along rights-of-way and in conifer plantations.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Glyphosate is generally nontoxic to a variety of invertebrate organisms. The formulation, Roundup® is toxic to some species due to the presence of toxic surfactants. The susceptibility of invertebrates and microorganisms to glyphosate toxicity exists over a broad range, with some species exhibiting effects at 3 ppm glyphosate and other species showing no effects at greater than 200 ppm. Studies conducted with various species of rainbow trout, bluegills, and other species of fish showed that these species were extremely tolerant to glyphosate. It has a low toxicity to birds.

The relatively low toxicity of glyphosate is reflected in acute oral LD_{50} values greater than 3,800 in mammals. Tests in rabbits indicate that the herbicide is not likely to pose a hazard by absorption through the skin. Patch tests of formulated product on human skin revealed no evidence of injury or sensitization to the herbicide. Tests in rabbits showed that Roundup® or glyphosate were only slightly irritating to the eyes. The irritation was completely reversible and no injury to the cornea was observed, indicating that with ordinary precautions glyphosate is not likely to cause injury to the eyes or cause blindness. Roundup® was only "slightly toxic", with an LC_{50} of 3.28 ppm when rats were exposed to an aerosol for 4 hours.

In subchronic 90-day oral studies, rats and dogs were fed glyphosate at concentrations of up to 2,000 ppm in the diet without any adverse effects. In chronic studies, rats and dogs were fed glyphosate in their diets at rates up to 300 ppm for 2 years, and mice were fed similar concentrations of glyphosate for 18 months. Except for some slight changes in the liver of rats (NOEL was 30 mg/kg/day), no significant treatment related abnormalities were observed. There was no evidence of carcinogenicity in studies with

rats and dogs. Studies in rats and rabbits showed that glyphosate is not teratogenic. There were no reproductive effects in rats when tested in multigeneration reproduction studies. Glyphosate was not mutagenic when tested in a dominant lethal mutation assay in mice or in a number of microbial mutagenicity tests employing bacteria or yeasts.

Environmental Fate

Glyphosate is absorbed by plant foliage and is then readily translocated throughout the plant to roots and rhizomes where it inhibits further growth and sprouting. It is not metabolized by plant tissue. Glyphosate has a very low lipid solubility and thus has little tendency to bioaccumulate in animals. Cows fed glyphosate had undetectable levels of residue in their milk.

Glyphosate is completely and rapidly degraded in soil by microbiological activity. In the soil environment, it is resistant to chemical degradation, stable to sunlight, is relatively nonleachable, has a low tendency to runoff, is strongly adsorbed to soil particles, has a negligible volatility, and has a minimal effect on soil microflora. Soil microflora degrade glyphosate to aminomethyl phosphonic acid which is somewhat more stable than glyphosate.

In aquatic systems, glyphosate is strongly adsorbed to both organic and mineral matter and is degraded primarily by microorganisms. The rate of degradation of glyphosate in water is generally slower than it is in most soils because there are fewer microorganisms in water than in most soils. Only very small amounts of applied glyphosate are removed in runoff.

Exposure and Hazard

Estimated occupational and environmental exposures to glyphosate from normal use are below those levels that would be expected to have acute or chronic toxic effects or reproductive effects. Glyphosate has not been shown to be mutagenic or carcinogenic at exposures well in excess of those to which forest workers, visitors, and animal residents would be exposed.

1.0 General Information

Glyphosate, also known by the trade names* Roundup® and Rodeo® for agricultural use, is a broad-spectrum, relatively nonselective herbicide first produced by the Monsanto Company in 1971 and first registered for use in 1974. Home-use products containing glyphosate are also available. Although the specific mode of herbicidal action of glyphosate is not entirely clear, it appears to inhibit synthesis of essential amino acids and to promote destruction of photosynthetic pigments in foliage (Shaner and Lyon 1980; Hoagland 1980). Within a week after application, treated plants yellow and wilt, and then turn brown and deteriorate (Monsanto Company 1982d). Glyphosate is absorbed primarily by the plant foliage, although minimal amounts of material may be taken up by roots under soil conditions that minimize glyphosate adsorption to soil particles. Foliar absorption is greatly increased by increased humidity and by the presence of surfactants in the formulation (Gottrup et al. 1976). Glyphosate is readily translocated to underground plant structures and is a strong inhibitor of sprouting in perennial species (U.S. Department of Energy 1983).

1.1 Normal Use Patterns

Glyphosate is used primarily in agricultural applications and for vegetation control in noncrop areas such as industrial and recreational areas, irrigation canals, and rights-of-way (Baird et al. 1971; Meister Publishing Company 1981; U.S. Department of Energy 1983). The use of glyphosate in forestry applications is relatively recent. It is used by such agencies as the USDA Forest Service, the Bureau of Land Management, and the Bonneville Power Authority (U.S. Department of Energy) for control of weeds and brush along rights-of-way and in conifer plantations. The quantities of glyphosate used by the USDA Forest Service for various applications in 1981 and 1982 are shown in Table 1-1.

In forest applications, glyphosate, as Roundup®, is applied either from the air or from the ground. Application rates are generally from 1.0 to 5.0 pounds active ingredient (a.i.) per acre (1.1 to 5.6 kg/ha) with the higher amounts used for site preparation and for noxious weed and poisonous plant control (Gross 1983). In general, control of annuals by glyphosate can be achieved with application rates of 0.3 to 1.0 lb a.i./acre (0.3 to 1.1 kg/ha) while perennials require rates of 1.0 to 4.0 lb a.i./acre (1.1 to 4.5 kg/ha) to achieve control (Weed Science Society of America 1983).

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service Use of Glyphosate

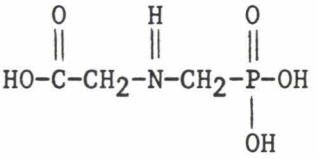
Purpose	Active Ingredient Per Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
1981					
Aerial					
Conifer release	1.0 to 2.0	1.5	6,627	Only liquid formulations are used.	Broadcast
Site preparation	1.0 to 2.0	1.6	1,416		
Ground					
Conifer release	1.0 to 2.0	1.0	1,034		
Site preparation	1.0 to 4.0	1.1	1,892	Only liquid formulations are used.	Broadcast
Range improvement	1.0	1.0	82		
Noxious weed and poisonous plant control	1.0 to 4.0	1.0	961		
1982					
Ground (103 projects)					
Timber, Range, Noxious Weeds, Nursery, Fire Management, Recreation, Engineering, Research, Right-of-way	0.1 to 5.0	1.9	13,906	Only liquid formulations are used.	Broadcast
Aerial (21 projects)					
Timber	1.25 to 4.0	2.0	6,540	Only liquid formulations are used.	Aerial

Source: Gross 1982.

Major formulations of glyphosate marketed in the United States are indicated in Table 1-2. Roundup® is glyphosate formulated with a proprietary surfactant. Rodeo® does not contain surfactant, but for its proper use the inclusion of a nonionic surfactant in the spray solution is recommended (Monsanto Company 1982d).

1.2 Physical and Chemical Properties

The active ingredient of glyphosate is N-(phosphonomethyl)glycine with the following structural formula:



It is a white, odorless solid with a melting point of 200°C (392°F). Pure glyphosate has a density of 0.5 g/cm^3. At 25°C (77°F), glyphosate has a solubility in water of 1.2 percent (12,000 ppm) but is not soluble in other solvents. Its vapor pressure is reported to be negligible. Glyphosate is corrosive to iron and to galvanized steel (Weed Science Society of America 1983).

Roundup® is a viscous, amber liquid with a specific gravity of 1.17 g/mℓ (water is 1.0 g/mℓ). It is completely miscible with water, is chemically stable, and has a shelf life of at least 4 years (Monsanto Company 1980, 1982b). Rodeo® is a clear, odorless, viscous, colorless to light amber liquid. It has a specific gravity of 1.22 to 1.25 g/mℓ and a slightly acidic pH (4.6 to 4.8). It is completely miscible with water and is chemically stable (Monsanto Company 1983a).

Table 1-2

Glyphosate Formulations for Forestry Use

Formulation	Active Ingredients (percent)	Active Ingredients (weight)	Inert Ingredients
Glyphosate Technical[a]	N-(phosphonomethyl) glycine. 95% or more (dry basis).	Not given	Water: 10 to 20%
Roundup®	41% isopropylamine salt of N-(phosphonomethyl) glycine.	Contains 480 grams per liter or 4 pounds per U.S. gallon of the isopropylamine salt. Equivalent to 356 grams per liter or 3 pounds per U.S. gallon of the acid, glyphosate.	Water, surfactants: 59.0%
Rodeo®	53.5% isopropylamine salt of N-(phosphonomethyl) glycine	Contains 648 grams per liter or 5.4 pounds per U.S. gallon of the isopropylamine salt. Equivalent to 480 grams per liter or 4 pounds per U.S. gallon of the acid glyphosate.	Water, other nondisclosed materials: 46.5% (manufacturer recommends use of 1 to 2 quarts of X-77 [Ortho] surfactant per 100 gallons of mixed spray solution).

[a]Glyphosate Technical is used for experimental applications such as toxicity testing and water and soil fate and persistence studies.

Source: Monsanto Company 1982a,b,d and 1983a.

2.0 Toxicity of Glyphosate

The toxicological properties of glyphosate, summarized in Table 2-1, show that this herbicide is generally nontoxic to a variety of organisms. However, when formulated as Roundup®, it is toxic to some animal species due to the presence of a surfactant. The surfactant is included to increase the rate of absorption of glyphosate by plants. Studies with rodents indicate that glyphosate is nonteratogenic; has little or no effect on fertility, reproduction, or development of offspring; is nonmutagenic; and appears to present no carcinogenic risk.

2.1 Invertebrates and Microorganisms

A variety of invertebrates (mostly arthropods) and microorganisms from freshwater, marine, and terrestrial ecosystems have been studied for toxic effects of technical glyphosate as well as formulated Roundup® and Rodeo®. Major findings of these studies are presented in Appendix A and indicate a broad range of susceptibility to glyphosate and its formulations in the organisms as tested. The increased toxicity of Roundup® compared to technical glyphosate in some studies results from the surfactant in Roundup® which is more toxic than glyphosate to some animal species. Appendices A and B include toxicity data on the surfactant alone.

For the most part, toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of glyphosate in a natural environment. For example, in the laboratory, aquatic organisms were kept for as long as 4 days in water containing dissolved glyphosate at various concentrations, and microorganisms were grown in a culture medium containing glyphosate. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to glyphosate or other man-made chemicals is usually a transient phenomenon depending on streamflow, chemical dissipation, and decomposition. Additionally, in natural systems, interactions with other man-made and naturally occurring chemicals, as well as changes in environmental factors such as temperature and moisture, all potentially affect herbicide assimilation and metabolism by invertebrates and microorganisms and thus alter their tolerance levels.

Among the insects tested (Appendix A), the LD_{50} for honeybees, representing insects of commercial value, was 100 mg/bee 48 hours after either ingestion of, or topical exposure to, Roundup® or to technical glyphosate. This level of experimental exposure is considerably in excess of exposure levels that would occur during

Table 2-1

Summary of Toxicological Properties of Glyphosate[a]

1. Acute Oral Toxicity

Organism (Formulation)	LD_{50} (mg/kg
Rats (Glyphosate)	4,320 to 5,600
(Roundup®)	4,900 to 5,400
Rabbits[b]	3,800
Fish (Glyphosate)	LC_{50} 16 to 168 ppm
(Roundup®)	LC_{50} 2.3 to 48 ppm
(Rodeo®)	LC_{50} >1,000 ppm
Birds (Glyphosate)	LC_{50} >4,640 ppm

2. Acute Dermal Toxicity

Organism (Formulation)	LD_{50} (mg/kg)
Rabbits (Glyphosate)	5,000
(Roundup®)	5,000

3. Subchronic Toxicity

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rats (technical)	90 days	200, 600, 2,000 ppm	None
Dogs (technical)	90 days	200, 600, 2,000 ppm	None
Hens	6 days	7,500 mg/kg body weight	None in neurotoxicity assay.

4. Chronic Toxicity

Organism	Length of Treatment	Dose Tested	Adverse Effects
Rats	2 years	30, 100, 300 ppm	None
Dogs	2 years	30, 100, 300 ppm	None

5. Dermal, Eye, and Inhalation Toxicity

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rabbits (Glyphosate)	Daily for 21 days	1x and 5x use level, dermal application	Practically non-irritating to the skin (score of 0.1 on scale of 8.0).

Table 2-1 (Continued)

5. Dermal, Eye, and Inhalation Toxicity (Concluded)

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rabbits (Roundup®)	Daily for 21 days	1x and 5x use level, dermal application	Moderately irritating to the skin (score of 4.3 on scale of 8.0).
Rabbits (Glyphosate)	One application to eye	Undiluted or 5% aqueous	Slightly irritating to eye. Score of 6.9 a scale of 110.
Rabbits (Roundup®)	One application to eye	Undiluted or 5% aqueous	Moderately irritating to eye. Score of 18.4 on a scale of 110.

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rats (Roundup®)	4-hour aerosol inhalation	--	Slightly toxic; LC_{50} is 3.28 ppm.
Human (Roundup®)	Patch test applied daily for 15 days	Undiluted	No evidence of injury or sensitization.

6. Teratology, Reproduction, and Feritilty

Organism	Treatment	Adverse Effects
Rabbits	Pregnant animals treated with 10 or 30 mg/kg body weight during period of organogenesis.	No teratogenic response.
Rats	Pregnant animals treated with 300, 1,000, and 3,500 mg/kg in days 6 through 19 of gestation.	No birth defects in offspring.
Rats	Three generation reproduction study. Fed diets containing 30, 100 or 300 ppm	Animals fed 300 ppm exhibited reduced mating, fertility and pregnancy only in the first litters born to the 2nd and 3rd generation animals. Not releated to treatment.

Table 2-1 (Concluded)

7. Mutagenicity

Organism	Test System	Dose Tested	Adverse Effects
Mice	Dominant lethal: male mice treated then mated to untreated females.	5 and 10 mg/kg	None
Bacteria	Microbial mutagenicity: 5 strains of *S. typhimurium*; 1 strain of *B. subtilis*; 1 strain of *E. coli*	Nonspecified	Nonmutagenic
Yeast	1 strain of *S. cerevisiae*	Nonspecified	Nonmutagenic

8. Carcinogenicity

Organism	Treatment	Adverse Effects
Mice	100 or 300 ppm in diet for 18 months	Noncarcinogenic
Rats	30, 100, 300 ppm in diet for 2 years	Noncarcinogenic
Dogs	30, 100, 300 ppm in diet for 2 years	Noncarcinogenic

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

[b]Where a specific formulation is not given, the original publication or source did not specify the formulation. Technical glyphosate is assumed to be the formulation used in these cases.

actual field applications of glyphosate (Monsanto Company data reported in Ghassemi et al. 1981).

Folmar et al. (1979) compared toxicities of Roundup®, glyphosate, and the proprietary surfactant incorporated in Roundup®, to midge larvae. They found that glyphosate alone, with an EC_{50}* of 55 ppm, was considerably less toxic than either Roundup® or the surfactant (18 ppm and 13 ppm, respectively).

Among the invertebrates studied, the median toxic levels for grass shrimp and fiddler crabs (marine crustaceans) were 281 and 934 ppm for technical glyphosate, respectively. There were no observable effects at 210 and 650 ppm when these crustaceans were exposed for 96 hours to technical glyphosate. For Daphnia, a freshwater crustacean, an LC_{50} of 192 ppm resulted from exposure for 48 hours to Roundup® (U.S. Department of Agriculture 1981). However, Folmar et al. (1979) found that 50 percent of Daphnia became immobilized (EC_{50}) at a concentration of 3 ppm of Roundup® after 48 hours of exposure.

Larval Atlantic oysters (Mollusca) were exposed for 48 hours to technical glyphosate at a concentration of 10 ppm. No effect was observed on subsequent embryonic development of the larvae (U.S. Department of Agriculture 1981).

The available information on the effect of glyphosate on soil microorganisms is somewhat ambiguous. Studies by Jaworski (1972) and Grossbard (1974), reviewed by Muller et al. (1981), indicated inhibition of microbial growth with glyphosate concentrations of 1.7 and 1.0 ppm, respectively. On the other hand, other studies have shown increased microflora populations at glyphosate soil levels as high as 8 ppm in sandy loam (Rueppel et al. 1977; Sacher 1978), no effect on soil nitrification at glyphosate concentrations of 300 ppm (Muller et al. 1981), no effect on nitrification and denitrification at 100 ppm (Torstensson 1978 in Muller et al. 1981), and no effect on amounts of ammonia and nitrate in soils with 100 ppm glyphosate (Marsh et al. 1977 in Muller et al. 1981). Finally, glyphosate applied to three soils (a silty clay loam, a sandy loam, and a silt loam) at 5 and 25 ppm, which reflect 1 and 5 times the normal application level, resulted in no apparent significant effects on microbial nitrogen fixation or nitrification. Additionally, there were no apparent effects on degradation of cellulose, starch, proteins, or leaf litter in this study (data from EPA registration files cited by Ghassemi et al. 1981). Section 3.2 of this report discusses the effects of different soil characteristics on adsorption and consequent biological

*EC_{50} (effective concentration) is the concentration at which 50 percent of the test organisms become immobilized.

inactivation of glyphosate. These physical factors of soil undoubtedly play an important role in the different observed effects of glyphosate on microbial activity in different studies and different soils.

2.2 Fish

Several studies have addressed the potential toxicity of glyphosate, Rodeo®, Roundup®, and the surfactant contained in Roundup®, on various fish species. These studies are summarized in Appendix B.

Clarke et al. (1970) propose that chemical substances for which the LC_{50} for fish is greater than 10 ppm be considered slightly toxic, that chemicals with an LC_{50} between 1 and 10 ppm be considered toxic, and that chemicals with an LC_{50} less than 1 ppm be considered highly toxic. For the most part, glyphosate alone is only slightly toxic to fish species with LC_{50}'s greater than 10 ppm. Roundup® and the surfactant used in Roundup® are generally toxic to fish, however, with LC_{50}'s less than 10 ppm. An exception is channel catfish, for which both Roundup® and glyphosate are only slightly toxic (Folmar et al. 1979; U.S. Department of Agriculture 1981).

Roundup® and the surfactant used in formulating Roundup® should be considered to be "harmful" or toxic to some species of fish, having 96 and 24 hour LC_{50}'s of 8.3 ppm or less (rainbow trout, fathead minnow, and bluegill) under static bioassay conditions (Folmar et al. 1979). It should be emphasized that in natural systems, the laboratory conditions of a static bioassay are highly unlikely and, as pointed out in Section 3.3 of this report, glyphosate does not accumulate readily in waterbodies through leaching and runoff from soil. Consequently, with normal application rates and practices, toxic levels (See Section 4.2.2.3) of residual glyphosate are unlikely to occur in waterbodies.

2.3 Birds

The toxic effects of glyphosate in birds have been investigated in a limited number of studies that are summarized in Appendix C. These studies, conducted in a laboratory environment, have included feeding studies of technical glyphosate, brief immersion of chicken eggs in Roundup®, and injection of technical glyphosate into 10-day old embryonated chicken eggs. In general, glyphosate appears to have low toxicity to avian species.

Eggs immersed in solutions of Roundup® at concentrations as high as 5 percent for 5 seconds showed no effect on hatchability or time to hatch when compared with control eggs (Batt et al. 1980). When both mallard ducks and bobwhite quail were fed technical glyphosate for 8 days, the LC_{50}'s were greater than 4,640 ppm (Monsanto Company 1982a). In studies with inbred chicken strains, an

LD_{50} of approximately 130 mg/kg was observed when glyphosate was injected into the air sac of 10-day old embryonated eggs, and an LD_{50} of approximately 250 mg/kg was observed when it was injected into yolk sacs (Olorunsogo et al. 1978).

In a neurotoxicity study with glyphosate in chickens, ten adult hens were dosed orally with 1,250 mg/kg, two-times daily, for three consecutive days. The regimen was repeated to give a cumulative dose of 15,000 mg glyphosate/kg. No behavioral or microscopic changes related to treatment were observed (Monsanto Company 1982a).

2.4 Mammals

Very few published reports on the toxicity of glyphosate in mammals appear in the open literature since almost all of the data are proprietary. Data have been provided by the Monsanto Company to the U.S Environmental Protection Agency as required by the Federal Insecticide, Fungicide, and Rodenticide Act, but much of these data are not available to the public. Summary data prepared by Monsanto appear in "Herbicide Bulletins" (Monsanto Company 1980, 1982a,b,c,d and 1983a,b), the Weed Science Society of America (1983), in U.S. Department of Agriculture (1981), and in a review by Wagner (1981). The U.S. Environmental Protection Agency has published no-observable-effect levels for glyphosate. The NOEL's for the following studies were: 90-day rat feeding, 2,000 ppm; 90-day dog feeding, 2,000 ppm; 18-month mouse feeding with no carcinogenic potential, 300 ppm; 2-year rat feeding, 100 ppm; 2-year dog feeding, 300 ppm; rabbit teratology, 30 mg/kg body weight; and three-generation rat reproduction, 300 ppm (U.S. Environmental Protection Agency 1978).

The following information is based on the above sources, a review by Newton and Dost (1981) and an earlier series of Herbicide Background Statements (U.S. Department of Agriculture 1981).

2.4.1 Acute Toxicity

Acute toxicity data have been reported for oral, dermal, inhalation and eye irritation studies.

2.4.1.1 Oral. Glyphosate, Roundup®, and Rodeo® are practically nontoxic in the animal species tested. The acute oral LD_{50} in rats is 5,600 mg/kg for glyphosate, 5,400 mg/kg for Roundup® and >5,000 mg/kg for Rodeo (Monsanto Company 1982a,b and 1983b). In rabbits, the LD_{50} for glyphosate is 3,800 mg/kg (U.S. Department of Energy 1983).

2.4.1.2 Dermal. When applied to the skin of rabbits, the minimal lethal dose was found to be greater than 7,940 mg/kg and the LD_{50} was greater than 5,000 mg/kg for both glyphosate and Roundup®. The LD_{50} for Rodeo® was >5,000 mg/kg when applied to the skin of rabbits (Monsanto Company 1982a,b and 1983a).

In a skin irritation test in rabbits, the Monsanto Company (1982a and 1983a) reported an FHSA (Federal Hazardous Substances Act) score of 0.1 for Rodeo® and glyphosate on a scale of 0 to 8.0, where 0 represents no irritation and 8.0 represents maximum irritation. For Roundup®, the reported FHSA score of 4.3 represents moderate irritation (Monsanto 1982b). In a human skin irritation (patch) test, no visible skin changes signifying reaction to injury were observed with Roundup (Monsanto Company 1982c).

2.4.1.3 Inhalation. Rats were exposed (up to 12.2 ppm Roundup®) to an aerosol for 4 hours. Upon sacrifice and autopsy either 10 or 14 days later, no treatment-related gross pathologies were found (U.S. Department of Energy 1983). The Monsanto Company (1982b), however, reported an inhalation LC_{50} of 3.28 ppm for Roundup® in rats exposed for 4 hours to an aerosol, indicating that the pesticide formulation is slightly toxic. A conservative position would assume the slight inhalation toxicity of this formulation.

2.4.1.4 Eye Irritation. Glyphosate, Roundup®, and Rodeo®, were only slightly irritating, moderately irritating, or nonirritating, respectively, when these substances were applied to the eyes of rabbits. On a scale that ranges from 0 (no effect) to 110 (maximum irritation), the reported FHSA scores for glyphosate, Roundup®, and Rodeo® were 6.9, 18.4, and 0.0, respectively (Monsanto Company 1982a,b and 1983a).

2.4.2 Subchronic Toxicity

2.4.2.1 Oral. Both beagle dogs and rats were fed glyphosate technical material at dietary levels of 200, 600, and 2,000 ppm for 90 days. No significant abnormalities were noted in the following parameters: weights, food consumption, behavioral reactions, mortality, hematology, blood chemistry, urologic studies, and gross and microscopic studies. Absolute organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios were not significantly different in treated and control dogs. Similarly, no abnormalities were noted when albino rats were fed glyphosate at the same dietary levels for 90 days. Complete gross and microscopic examinations of organs and tissues from all animals revealed no lesions in organs or tissues that could be related to treatment effect (U.S. Department of Agriculture 1981; Weed Science Society of America 1983).

2.4.2.2 Dermal. In a 21-day dermal study, rabbits were treated daily with Roundup diluted to its use level and to 5 times its use level. These early studies suggested that repeated applications of Roundup® produced several skin irritations. The skin irritation and resulting marked reduction in food consumption contributed to the presence of testicular atrophy which was observed in the test

animals. Testicular atrophy which was also observed in the control animals was considered due to result of stress on the animals. Further study found that the skin reactions were due to the surfactant in the product. In a human patch test, Roundup® was applied to the skin of 50 subjects daily for 15 days. No visible skin changes signifying reaction to injury were observed in any of the subjects. When a challenge application was later made to detect skin sensitization, no visible skin changes were observed. The formulation, therefore, was not a primary irritant or skin sensitizer (U.S. Department of Agriculture 1981). The product, therefore, does not present a handling hazard since the negative human exposure data takes precedence over the rabbit experiments.

2.4.3 Chronic Toxicity

In a long-term feeding study, groups of 50 male and 50 female rats were fed 30, 100, or 300 ppm glyphosate in their diet for 2 years. There were no significant differences in food consumption, weight gains, or final body weights among the test and control groups. Hematological, clinical, chemical, and urine analyses conducted at 3, 6, 9, 12, 15, 18, and 24 months on animals fed 300 ppm revealed no abnormalities (U.S. Department of Agriculture 1981). Complete gross and microscopic examinations were conducted on organs and tissues from the control and treated animals. There were no gross or histopathologic evidence of any difference in frequency or type of tumors that could be related to treatment. Histopathologic examination revealed a treatment-related increase in lipid content in the liver of animals fed 300 ppm. These changes were not present in the livers from rats fed 30 or 100 ppm.

Groups of 4 male and 4 female dogs were fed 0, 30, 100, or 300 ppm glyphosate in their diet for 2 years. There were no consistent differences between values in the control and treatment groups. Absolute organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios revealed no significant differences between test and control dogs. No gross or histopathologic changes were observed in the livers of dogs fed 30, 100, or 300 ppm for 2 years (U.S. Department of Agriculture 1981).

No cellular changes (increased incidence of cytoplasmic vacuoles or lipid content) were observed in mice fed glyphosate at a concentration up to 300 ppm in the diet for 18 months (U.S. Department of Agriculture 1981).

2.4.4 Reproductive and Teratogenic Effects

No treatment related effects in parental or pup body weight gain, behavior, survival, or reproductive performance were observed in a

three-generation reproduction study in which rats were fed glyphosate at dosages of 3, 10, and 30 mg/kg body weight (30, 100, and 300 ppm). Gross pathologic examination revealed no differences between treated and control animals. Organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios revealed no consistent differences. Histopathologic examination revealed no treatment-induced lesions. No other adverse effects on reproductive ability were noted except for slightly reduced mating indices in high dose females of the third generation only, and slightly reduced pregnancy rates at all treatment levels of the first generation only. These changes were not considered treatment-related. Gross and microscopic examination of randomly selected offspring of the third generation revealed no significant differences between treated and control groups (Monsanto Company study reported in U.S. Department of Agriculture 1981).

Pregnant rats were treated with glyphosate at dosages of 300, 1,000, and 3,500 mg/kg/day on days 6 through 19 of gestation, the period of organogenesis. No evidence of birth defects in the offspring was observed (Monsanto Company 1982a). Treatment of pregnant albino rabbits with dose levels of 75, 175, and 350 mg/kg body weight on days 6 through 27 of gestation did not induce a teratogenic response (Monsanto Company 1982a).

Several additional reproductive studies with glyphosate are reported in Appendix D.

2.4.5 Carcinogenicity

In an 18-month carcinogenicity study, Swiss mice were fed glyphosate at dietary levels of either 100 or 300 ppm. Gross and microscopic pathologic examinations revealed no correlation between treatment and tumor incidences in the treated mice. A carcinogenic response was not induced. The incidence or pattern of mortalities was not significantly different between treatment and control groups (Monsanto Company data reported in U.S. Department of Agriculture 1981). Similar results have been reported in rats and dogs fed glyphosate at dietary levels up to 300 ppm for 2 years (U.S. Department of Agriculture 1981). In a more recent lifetime study (Monsanto Company 1983b), no carcinogenic effects were observed when glyphosate was fed to rats at dosages up to 31 mg/kg (equivalent to about 600 ppm in adult rats).

2.5 Mutagenicity

Glyphosate is not a mutagen. Male mice were given 200, 800, or 2,000 mg glyphosate/kg body weight and subsequently mated with untreated females. No evidence of mutagenicity was observed in this

dominant lethal mutation assay, indicating that the test material is not genotoxic to germ cells (Monsanto Company 1982a).

Three different types of microbial mutagenicity tests were performed using glyphosate (Monsanto Company 1982a). In these studies, a total of eight strains (7 bacterial and 1 yeast) including five strains of Salmonella typhimurium and one strain each of Bacillus subtilis, Escherichia coli, and Saccharomyces cerevisiae (yeast) were treated. No mutagenic effect was reported in any of these strains.

3.0 Environmental Fate

Glyphosate is an effective herbicide for a broad-spectrum of plant species, yet it has short persistence in soil and water and has relatively low toxicity to animal species. Glyphosate is taken up by the plant primarily through the foliage and is then translocated through the plant to roots and other structures. Of the glyphosate in the soil, less than 1 percent is absorbed through the roots, primarily because glyphosate is quickly and tightly adsorbed to soil particles. Glyphosate is not metabolized in plants, although complete and rapid degradation occurs in both soil and water by microbiological activity but not by chemical activity (Rueppel et al. 1977, Ghassemi et al. 1981).

3.1 Bioaccumulation

3.1.1 Plants

The major pathway for uptake of glyphosate in plants is through the foliage, although, depending upon soil type and conditions, some root uptake may occur. Gottrup et al. (1976) demonstrated that increased humidity and the presence of surfactants increased the rate of absorption of glyphosate by plant foliage. Foliar absorption experiments have indicated that the surfactants serve to increase the rate of diffusion of glyphosate across the plant plasma membrane rather than across the cuticle (Wyrill and Burnside 1976). Once the glyphosate is taken up by the plant tissue, it is translocated throughout the plant, including through underground structures such as roots and rhizomes where it inhibits further growth and sprouting. Glyphosate is stable within the plant tissue and is not metabolized by plants (Gottrup et al., 1976; Wyrill and Burnside 1976).

3.1.2 Animals

Glyphosate has a very low octanol/water partition coefficient (0.17×10^{-2} at 20 ppm and 0.6×10^{-3} at 100 ppm) indicating its low solubility in lipids. Thus it has little tendency to bioconcentrate in animals (Monsanto Company data cited in Ghassemi et al. 1981). Residue and metabolism studies have indicated that glyphosate is only slowly absorbed across the gastrointestinal membranes and that, in vertebrates tested, there is minimal tissue retention and rapid elimination of residues (Monsanto Company 1982a). Data on the metabolism of glyphosate in animals are summarized in Appendix F.

Radiolabelled glyphosate fed in a single dose to rabbits was largely excreted in feces (>80 percent) or urine (7 to 11 percent) within five days, with small amounts (<1 percent) expired as CO_2 or remaining in the colon (U.S. Environmental Protection Agency data reported by Ghassemi et al. 1981). In bluegill fish exposed to

0.612 ppm radiolabelled glyphosate for 28 days, there was an accumulation of residue in edible portions of the fish with a bioconcentration factor of 1.6 (U.S. Department of Agriculture 1981). Sacher (1978), however, reported maximum residue levels in fish tissue of 0.55 ppm for channel catfish sampled 7 days after the fish were exposed to 10 ppm glyphosate for 14 days. Largemouth bass and rainbow trout exposed to 10 ppm for 14 days had maximum residue levels of 0.12 and 0.11 ppm, respectively. Rainbow trout exposed to varying concentrations of glyphosate for 12 hours had no detectable residues of glyphosate or its primary metabolite (aminomethyl phosphonic acid). However, trout exposed to Roundup at 2.0 ppm for 12 hours were reported to have 80 ppm of glyphosate in fillets and 60 ppb of glyphosate in eggs (Folmar et al. 1979). The reported 80 ppm glyphosate residue in trout fillets is probably a typographical error, since such a figure would yield a bioaccumulation factor considerably in excess of others reported for glyphosate. A likely correct figure for the trout fillet residues is 80 ppb glyphosate (Peterson 1984). Monsanto Company (1983b) reports bioconcentration factors of 0.1 to 0.3.

Cows fed glyphosate had undetectable levels of glyphosate residues (<0.025 ppm) in milk (Monsanto Company 1982c). Chickens were found to have a bioaccumulation factor as low as 10^{-4} for glyphosate in various tissues (Sacher 1978). No storage of radiolabelled glyphosate was found in muscle or fat of bobwhite quail, although traces were found in liver and kidney tissue (U.S. Department of Agriculture 1981). Folmar et al. (1979) were unable to detect residues of glyphosate or Roundup® in midge larvae exposed to either substance at concentrations as high as 2.0 ppm.

3.2 Soil

Glyphosate is completely and rapidly degraded in soil by microbiological activity. In the soil environment it is resistant to chemical degradation, is stable to sunlight, is relatively nonleachable, has a low tendency to runoff, is strongly adsorbed to soil particles, has a negligible volatility, and has a minimal effect on soil microflora (Rueppel et al. 1977; Weed Science Society of America 1983). The tendency of glyphosate to adsorb to soil particles influences the potential for leaching, runoff, and degradation by soil microorganisms--all of which are major factors that determine the rate of dissipation of glyphosate from the soil.

Studies by Rueppel et al. (1977) have demonstrated the resistance of glyphosate to photodecomposition. A solution of radiolabelled glyphosate was irradiated for 48 hours in a Crosby photoreactor with no loss of ^{14}C content due to volatile degradation products and no observed changes in the composition of the test solution.

Sacher (1978) compared $^{14}CO_2$ evolution over a period of seven days in sterile and nonsterile conditions with radiolabelled glyphosate and with radiolabelled sucrose (sugar) as a control. Minimal amounts of $^{14}CO_2$ evolved, along with lack of significant changes in composition of the radiolabelled glyphosate compounds under sterile conditions, demonstrated that chemical degradation is not a major means of glyphosate degradation. The importance of biodegradation by soil microflora was indicated in the nonsterilized conditions, in which as much as 55 percent of the ^{14}C-labelled glyphosate was given off as $^{14}CO_2$ within 4 weeks using Lintonia Sandy Loam soil. Microbial degradation of glyphosate can occur under both aerobic and anaerobic conditions and the same general distribution of glyphosate metabolites is found under both aerobic and anaerobic conditions (Rueppel et al. 1977).

The primary metabolite of glyphosate is aminomethyl phosphonic acid (AMPA). Studies with radiolabelled AMPA in silt loam and silty clay loam soils resulted in 34.8 and 16.1 percent of the applied ^{14}C being given off as $^{14}CO_2$ within 63 days, respectively (Rueppel et al. 1977). Degradation of AMPA is generally slower than that of glyphosate, possibly because AMPA may adsorb onto soil particles more strongly than glyphosate and/or because it may have a lower permeability through the cell walls or membranes of soil microorganisms.

Glyphosate is relatively immobile in most soil environments as a result of its strong adsorption to soil particles. This tendency of glyphosate to adsorb to soil particles serves as the initial stage in the inactivation of glyphosate with respect to plant uptake--the adsorbed glyphosate is unavailable for uptake by plant roots. Adsorption of glyphosate to soil particles begins immediately after application, and binding occurs with particular rapidity to kaolinite, illite, and bentonite clays as well as to muck. Organic matter and clays saturated with Fe^{+++} and Al^{+++} tend to adsorb more glyphosate than do organic matter and clays saturated with Na^{+} or CA^{++}. The prime factor in determining the amount of glyphosate adsorbed to soil particles is the soil phosphate level, and it appears that glyphosate is bound to soil through the phosphonic acid moiety (Sprankle et al. 1975a).

The effect of both pH and phosphate on glyphosate adsorption was examined by Sprankle et al. (1975b) based on plant yield as an indicator of glyphosate unavailability due to adsorption. They found no significant difference in plant yield with differing soil pH's at glyphosate application rates up to 9.8 lb/acre (11 kg/ha), but at 50 lb/acre (56 kg/ha), there was a trend for decreased yields at lower pH (4.6 to 5.6) compared to higher pH (6.1 to 6.7). These data indicate a decrease in glyphosate adsorption as the pH is raised; however, glyphosate is usually applied at the rate of 1.0 to

4.0 lb/acre (1.12 to 4.6 kg/ha). In terms of phosphate, glyphosate experimentally applied at 10 lb/acre (11.2 kg/ha) with 88 lb/acre (98 kg/ha) of phosphate resulted in significant reduction in yield while 10 lb/acre (11.2 kg/ha) of glyphosate application without phosphate resulted in no effect on plant yield. Thus, high levels of phosphate resulted in low adsorption of glyphosate to soil. The initial binding of glyphosate to soil appears to be reversible, with phosphate anions competing with glyphosate for binding sites, due to anion exchange in the soil matrix.

Salazar and Appleby (1982), however, found that in some high-organic soils, glyphosate applied to soil at a rate of 3 lb/acre (3.4 kg/ha) reduced bentgrass seedling growth for seeds germinating as long as 5 days after glyphosate application. Brewster and Appleby (1972 in Salazar and Appleby 1982) found similar reduction in wheat seedling growth with pre-emergence application of glyphosate that appeared to be related to increased levels of moisture in the soil.

Several studies have shown that glyphosate does not adversely affect soil microorganisms or their metabolic processes. Muller et al. (1981) studied the effect of glyphosate application at 2.3 lb/acre (2.6 kg/ha) in a loam soil and a fine silt soil in Finland during actual agricultural applications. They concluded that glyphosate was degraded over the winter months even at the low prevailing temperatures and that glyphosate did not adversely affect nitrogen fixation, nitrification, or denitrification activity in these soils. The relatively benign nature of glyphosate with respect to soil microorganisms is further confirmed by tests conducted by Monsanto personnel who compared microbial plate counts on untreated soil and soil treated with glyphosate (Sacher 1978). Results of glyphosate tests with soil microorganisms are presented in Appendix A.

In general, glyphosate dissipates relatively rapidly when applied to most soils. As a result of greenhouse soil dissipation studies with three different soil types, Rueppel et al. (1977) calculated half lives from 3 to 130 days, the former for a silty clay loam with 6 percent organic content and the latter for a sandy loam with 1 percent organic content. These data have been substantiated by field studies, in which glyphosate had an average half-life of 2 months in 11 soils and by other studies reporting half-lives of 17 to 19 weeks in sandy soil and 3 weeks in silt loam (U.S. Environmental Protection Agency data reported in Ghassemi et al. 1981).

3.3 Water

In aquatic systems, glyphosate is strongly adsorbed to both organic and mineral matter and is degraded primarily by microorganisms. However, the rate of degradation of glyphosate in water is generally

slower than it is in most soils because there are fewer microorganisms in water than in most soils (Ghassemi et al. 1981).

Stream samples of flowing canal water were taken by Comes et al. (1976) following metering of Roundup® at a rate of 150 ppb of glyphosate into the canals. Sampling 1 mile (1.6 km) downstream accounted for only 70 to 72 percent of the total glyphosate introduced into the stream. Subsequent downstream disappearance of glyphosate diminished and 5 to 9 miles (8 to 14.4 km) downstream, only 57 to 58 percent of the glyphosate remained in the water flow. These figures are for herbicide metered directly into flowing water. Sacher (1978) reports that when glyphosate is applied to ditch banks at 150 ppb concentration, maxima of only 10 and 3 ppb glyphosate could be expected in streamflow 1 and 5 miles (1.6 and 8 km) downstream, respectively.

In a pond at Fort Lauderdale, Florida, Sacher (1978) reported a half-life for glyphosate of approximately 12 days. Studies by Monsanto Company (U.S. Environmental Protection Agency data reported in Ghassemi et al. 1981) on glyphosate persistence in natural water bodies found a half-life of 7 weeks in Sphagnum bogs at pH 4.23; of 9 weeks in cattail swamps at pH 6.25; and of 10 weeks in pond water at pH 7.33.

As a result of the strong adsorption of glyphosate to soil particles, its mobility due to leaching and surface washout is greatly reduced. Rueppel et al. (1977) tested the mobility of glyphosate in three different soils by means of soil thin-layer plates spotted with radiolabelled glyphosate. These plates were then washed twice with water and the final distribution of radiolabelled glyphosate determined by beta camera analysis after each washing. On all three soils tested, the parent glyphosate, even after the second washing, moved only a short distance, indicating that glyphosate is an immobile pesticide.

Leaching of residues from irrigation canal banks treated with glyphosate was investigated by Comes et al. (1976). Neither glyphosate nor its metabolite, aminomethyl phosphonic acid, were detected in the first flow of water through canals that had been dry for 23 weeks after glyphosate had been sprayed on the ditch banks at a rate of 5 lb/acre (5.6 kg/ha). Soil samples collected from the canal bed the day before the canals were filled indicated residual levels of both glyphosate and aminomethyl phosphonic acid (0.35 and 0.78 ppm, respectively) in the top 10 centimeters of the soil along the ditch banks. The authors concluded that glyphosate could be applied to ditch bank vegetation in the fall after draining canals, with little to no chance of glyphosate residues contaminating irrigation water the following spring.

The Monsanto Company (U.S. Environmental Protection Agency data reported in Ghassemi et al. 1981) conducted soil column leaching studies in which glyphosate was aged for 30 days in soil columns and

then eluted for 45 days with 1/2 acre-inch of water. Leaching of parent compound was found to be insignificant. Inclined beds of three different soils were used to evaluate the runoff potential of glyphosate (Rueppel et al. 1977). Radiolabelled glyphosate was applied uniformly at a rate of 1.0 lb/acre (1.12 kg/ha) to the upper third of the soil bed and the surface then subjected to artificial rainfalls at days 1, 3, and 7 after treatment. The artificial rainfall was continued long enough for the collection of two consecutive 50 ml samples of runoff water and sediment. The ^{14}C activity of runoff water and sediment was measured. The results indicated a maximum runoff rate of less than 1.8×10^{-4} lb/acre (2×10^{-4} kg/ha), confirming that glyphosate binds tightly to soil particles.

Studies of the runoff of Roundup® applied to agricultural soils in Ohio were conducted by Edwards et al. (1980). Roundup® was applied as a pre-seeding treatment in early spring at rates of 1, 3, and 8 lb/acre (1.10, 3.36, and 8.96 kg/ha) and levels of glyphosate in the runoff water from natural rainfall were measured. The highest concentration of residual glyphosate, 5.2 ppm, was contained in runoff 1 day following treatment at the highest application rate. Glyphosate levels up to 2 ppb were detected in runoff from the highest application rate for up to 4 months following treatment. For watersheds treated at lower application rates, the highest level of glyphosate detected in runoff was 100 ppb for rainfall events 9 to 10 days after treatment. Two months following treatment, residual glyphosate levels in runoff had decreased to 2 ppb. Of the glyphosate applied to the soil, a maximum of 1.85 percent was removed by runoff transport and, of this amount, 99 percent was removed during the first rainfall event after herbicide application.

3.4 Air

The vapor pressure of glyphosate is negligible (Ghassemi et al. 1981, U.S. Department of Energy 1983). Rueppel et al. (1977) report that only small amounts of glyphosate should be expected to volatilize from either soil or water.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to glyphosate during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved glyphosate. Calculations of exposure to glyphosate in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to glyphosate are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational doses, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Glyphosate Application

Available application data for glyphosate use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to glyphosate differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of glyphosate in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Glyphosate is rapidly adsorbed to soil particles and to sediment in ponds and streams. It is also rapidly degraded. Consequently, the potential for incidental exposure of humans and wildlife to glyphosate is low, existing only for a very short period immediately after its application.

4.2 Exposure

Estimates of potential exposure to glyphosate are based on aerial as well as ground application. The maximum aerial application rate for a glyphosate (see Table 1-1) is 4.0 lb a.i./acre, while the compound is applied from the ground (ground foliar) at rates as high as 5 lb a.i./acre. In both aerial application and ground foliar

Table 4-1

USDA Forest Service Application Data for Glyphosate (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Glyphosate (103)	Ground Foliar	4.9	505	5.4	8.2	7.0	9.8	Timber (48), Range (17) Noxious Weeds (12) Nursery (7), Fire (1) Recreation (3) Engineering (1) Research (12) Right-of-Way (2)
Glyphosate (21)	Aerial	8.4	176	4.7	3.5	6.8	4.5	Timber (21)

[a]Grand Total = 681

Source: Gross 1983

application, glyphosate is applied as a liquid. The calculations in Table 4-2 that are based on aerial application (e.g., spray/observer dose, wildlife dermal exposures, and water concentrations) assume an application rate of 4.0 lb a.i./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g, backpack sprayer dose, and wildlife oral exposures) assume an application rate of 5.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of glyphosate and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of glyphosate to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for glyphosate are based on exposures on a per pound per acre application rate multiplied by 4 lb/acre for observers and 5 lb/acre for backpack sprayers. In the case of the aerial spray observer, exposure estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. However, for glyphosate, the rate of absorption is assumed to be 1 percent. The lower glyphosate absorption rate is used for this assessment because the very low octanol/water partition coefficient of glyphosate (Section 3.1.2) indicates its probable low skin penetration. At 4.0 lb a.i./acre application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.016 mg/kg (0.004 mg/kg at 1 lb/acre application rate since dermal absorption is 1 percent rather than 10 percent).

For ground applications at 5 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.20 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Glyphosate

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg[b]		
Observer	up to 0.016 mg/kg		
Backpack Sprayer	0.20 mg/kg		
Environmental Exposure			
Fish	0.04 ppm (Immersion)[c]		
Rabbits	1.612 mg/kg	Negligible	5.10 mg/kg
Deer	0.404 mg/kg	Negligible	7.20 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.0008 mg/kg (2 days)
(Rabbits)	NA	NA	0.021 mg/kg (2 days)
(Deer)	NA	NA	0.029 mg/kg (2 days)
(Water)	NA	NA	0.0016 mg/kg (1 day)
(Berries)	NA	NA	0.004 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0020 mg/kg (1 day)
Toxicity Summary	Acute LD_{50} >5,000 mg/kg in rabbits with glyphosate and Roundup®	LC_{50} = 3.28 ppm in rats with Roundup®	Acute LD_{50}'s of 3,800 to 5,600 for glyphosate in rats and rabbits, respectively. Rat lifetime NOEL >31 mg/kg for glyphosate. ADI (human) of 0.05 mg/kg.
	Most studies, LC_{50} >10 ppm for fish		

[a]Assumes aerial application at 4 lb active ingredient per acre and ground foliar application at 5 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure on a daily basis.

[c]Fish exposure results from immersion in water containing glyphosate.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by glyphosate application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less (by orders of magnitude) than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 4 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 1.612 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.404 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of glyphosate vapor would be negligible since the vapor pressure of glyphosate is reported to be negligible (Weed Science Society of America 1983).

Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of glyphosate in water, calculated as:

Water concentration: 0.04 ppm (0.01 ppm at 1 lb/acre application rate)

This concentration of glyphosate represents the estimated maximum concentration immediately after spraying. If heavy rainfall occurred immediately after spraying, higher transient concentrations could occur in streams and ponds. Edwards et al. (1980) found 5.2 ppm in runoff from agricultural fields when application of 8 lb/acre as Roundup® was followed by precipitation within one day. However, such high concentrations result from precipitation soon after application, and would be transient since they are rapidly diluted by resident waters.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of glyphosate by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Maximum reasonable oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 5.0 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 7.20 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 5.10 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of glyphosate in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.04 ppm

- bioconcentration: 1.0 (actual bioconcentration for glyphosate is < 1.0 in most studies) (Sacher 1978, Monsanto 1983b)

Fish tissue concentration: 0.04 ppm (0.04 mg/kg)

Deer and rabbit tissue concentrations include glyphosate accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied glyphosate is absorbed at a rate of 1 percent for reasons discussed in Section 4.2.1 with regard to spray-observer absorption rates. It is also assumed that oral exposure of deer and rabbit to glyphosate lasts only for 2 days due to the relatively short environmental persistence of glyphosate. Deer tissue concentrations of glyphosate are derived as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 0.404 mg/kg (dermal exposure) x 0.01 = 0.004 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 7.20 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.440 mg/kg.

Total Deer Tissue concentration = 0.004 mg/kg + 1.440 mg/kg = 1.444 mg/kg (1.444 ppm).

Rabbit tissue concentrations of glyphosate are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 1.612 mg/kg (dermal exposure) x 0.01 = 0.016 mg/kg

Oral Exposure contribution assumes 10 percent absorption of two-days feeding, or 5.10 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.020 mg/kg.

Total Rabbit Tissue Concentration = 0.016 mg/kg + 1.020 mg/kg = 1.036 mg/kg (1.036 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to glyphosate from eating meat are derived:

Fish meat: 0.040 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0008 mg/kg

Deer meat: 1.444 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.029 mg/kg

Rabbit meat: 1.036 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.021 mg/kg

Water and plant consumption by man would result in the following oral exposures to glyphosate based on assumptions stated in the General Introduction to the Herbicide Background Statements at an aerial application rate of 4 lb a.i./acre:

Water:	0.0016 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries:	0.004 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms:	0.0020 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of glyphosate to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational dose and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rat inhalation results, rabbit and rodent acute oral LD_{50}'s, rat chronic oral NOEL, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for USDA Forest Service applications. Based upon chronic feeding studies, NOEL's of 31 mg/kg are reported in rats. Assuming a 100-fold margin of safety for extrapolation to man (a factor of 10 for inter-species variability and a factor of 10 for human intra-species variability), one can assume that 31 mg/kg ÷ 100, or a 0.31 mg/kg oral dose in man, will not result in adverse acute effects. The estimated human exposures from ingestion of water, game, and plant material are well below 0.31 mg/kg per day.

As a more stringent limitation, the U.S. Environmental Protection Agency has promulgated an acceptable daily intake (ADI) of 0.05 mg/kg body weight/day for glyphosate in the human diet (U.S. Environmental Protection Agency 1982). Ingestion of wildlife, fish, and plant material containing glyphosate as estimated (Table 4-2) should not result in consumption of glyphosate by humans that is in excess of the ADI. Furthermore, the ADI is for an average lifetime dose, where levels as estimated here would be only temporary, lasting for periods on the order of days.

Small mammal studies have shown that glyphosate applied to the eye is slightly to mildly irritating and that glyphosate fed at rates up to 300 ppm in feed does not result in birth defects (in rats). Glyphosate is not mutagenic and does not appear to be carcinogenic. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from glyphosate use, if proper care is taken during application.

Appendix A

Summary of Glyphosate Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	References
Formulated Product (Roundup®) and Technical Product (Glyphosate)	Honeybees (Apis mellifera; Insecta)	Ingestion Surface absorption	48 hours 48 hours	LD_{50} = 100 mg/bee LD_{50} = 100 mg/bee	--[b] --	Monsanto Company data cited in Ghassemi et al. 1981
Glyphosate	Midge larvae (Chironomus plumosus; Insecta)	Surface absorption	48 hours	EC_{50} = 55 ppm	(31-97 ppm) 22°C (72°F)	Folmar et al. 1979
Surfactant used in Roundup®	Midge larvae (Chironomus plumosus; Insecta)	Surface absorption	48 hours	EC_{50} = 13 ppm	(7.1-24 ppm) 22°C (72°F)	
Roundup®	Midge larvae (Chironomus plumosus; Insecta)	Surface absorption	48 hours	EC_{50} = 18 ppm	(94-32 ppm) 22°C (72°F)	Folmar et al. 1979
Glyphosate, Technical	Grass shrimp (Crustacea)	Surface absorption	96 hours	TL_{50} = 281 ppm NOEL at 210 ppm	(207-391 ppm)	Monsanto Company 1982 and U.S. Department of Agriculture 1981
Glyphosate, Technical	Fiddler crab (Crustacea)	Surface absorption	96 hours	TL_{50} = 934 ppm NOEL at 650 ppm	(555-1,570 ppm)	
Formulated Product (Roundup®)	Cladoceran Daphnia sp.; Crustacea)	Surface absorption	48 hours	LC_{50} = 192 ppm	(181-205 ppm)	U.S. Department of Agriculture 1981
Roundup®	Cladoceran Daphnia sp.; (Crustacea)	Surface absorption	48 hours	LC_{50} = 5.3 ppm	--	Monsanto Company 1982
Roundup®	Cladoceran (Daphnia magna; Crustacea)	Surface absorption	48 hours	EC_{50} = 3.0 ppm	(2.6-3.4 ppm) 22°C (72°F)	Folmar et al. 1979
Rodeo®	Cladoceran (Daphnia sp.; Crustacea)	Surface absorption	48 hours	--	LC_{50} = 930 ppm	Monsanto Company 1982d

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	Rererences
Roundup®	Amphipod (Gammarus pseudolimnaeus; Crustacea)	Surface absorption	48 hours 96 hours	LC_{50} = 62 ppm LC_{50} = 43 ppm	(40-98 ppm) 12°C (54°F) (28-66 ppm) 12°C (54°F)	Folmar et al. 1979
Roundup®	Crayfish (Crustacea)	Surface absorption	96 hours	LC_{50} >1,000 ppm	--	Monsanto Company 1982b
Roundup®	Harpacticoid (Nitocra spinipes; Crustacea)	Surface absorption	96 hours	LC_{50} = 22 ppm	(17-29 ppm) 21•1°C (70 ± 2°F)	Linden et al. 1979
Glyphosate, Technical	Cladoceran (Daphnia sp.; Crustacea)	Surface absorption	40 hours	LC_{50} = 780 ppm	--	Monsanto Company 1982a
Glyphosate, Technical	Larval Atlantic oysters (Mollusca)	Surface absorption	48 hours	NOEL at 10 ppm	No effect on embryonic development of larvae.	Monsanto Company 1982a and U.S. Department of Agriculture 1981
Glyphosate	Bacterium (Rhizobium japonicum)	Surface absorption	--	Growth inhibited at 1.7 ppm concentration of glyphosate.	--	Jaworski 1972 in Muller et al. 1981
Glyphosate	Stachybotrys chartarum	Surface absorption	--	Inhibited at 1.0 ppm concentration of glyphosate	--	Grossbard 1974 in Muller et al. 1981

[a]Values in parentheses are the 95% confidence limits.

[b]Data not available or not applicable.

Appendix B

Summary of Glyphosate Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	References
Roundup®	Rainbow trout	Static	24 hours	LC_{50} = 8.3 ppm	(7.0-9.9 ppm) 12°C (54°F)	Folmar et al. 1979
		bioassay	96 hours	LC_{50} = 8.3 ppm	(7.0-9.9 ppm) 12°C (54°F)	
Roundup®	Fathead minnow	Static	24 hours	LC_{50} = 2.4 ppm	(2.0-2.9 ppm) 22°C (72°F)	
		bioassay	96 hours	LC_{50} = 2.3 ppm	(1.9-2.8 ppm) 22°C (72°F)	
Roundup®	Channel catfish	Static	24 hours	LC_{50} = 13 ppm	(11-16 ppm) 22°C (72°F)	
		bioassay	96 hours	LC_{50} = 13 ppm	(11-16 ppm) 22°C (72°F)	
Roundup®	Bluegill	Static	24 hours	LC_{50} = 6.4 ppm	(4.8-8.6 ppm) 22°C (72°F)	
		bioassay	96 hours	LC_{50} = 5.0 ppm	(3.8-6.6 ppm) 22°C (72°F)	
Roundup®	Bluegill	--	96 hours	TL_{50} = 14 ppm	--[b]	
Roundup®	Carp	--	96 hours	TL_{50} = 3.9 ppm	--	
Roundup®	Trout	--	96 hours	TL_{50} = 11 ppm	--	Monsanto Company 1982b
Roundup®	Catfish	--	96 hours	LC_{50} = 16 ppm	--	
Roundup®	Fatheat minnow	--	96 hours	LC_{50} = 9.4 ppm	--	
Formulated Product (Roundup®)	Rainbow trout	--	96 hours	TL_{50} = 48 ppm	--	
Formulated Product (Roundup®)	Bluegill	--	96 hours	TL_{50} = 24 ppm	--	U.S. Department of Agriculture 1981
Rodeo®	Carp	--	96 hours	TL_{50} >10,000	--	
	Trout	--	96 hours	LC_{50} >1,000	--	Monsanto Company 1982d
	Bluegill	--	96 hours	LC_{50} >1,000	--	
Glyphosate	Rainbow trout	Static	24 hours	LC_{50} = 140 ppm	(120-170 ppm) 12°C (54°F)	
		bioassay	96 hours	LC_{50} = 140 ppm	(120-170 ppm) 12°C (54°F)	
Glyphosate	Fathead minnow	Static	24 hours	LC_{50} = 97 ppm	(79-120 ppm) 22°C (72°F)	Folmar et al. 1979
		bioassay	96 hours	LC_{50} = 97 ppm	(79-120 ppm) 22°C (72°F)	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	References
Glyphosate	Channel catfish	Static bioassay	24 hours 96 hours	LC_{50} = 130 ppm LC_{50} = 130 ppm	(110-160 ppm) 22°C (72°F) (110-160 ppm) 22°C (72°F)	Folmar et al. 1979
Glyphosate	Bluegill	Static bioassay	24 hours 96 hours	LC_{50} = 150 ppm LC_{50} = 150 ppm	(120-190 ppm) 22°C (72°F) (120-190 ppm) 22°C (72°F)	
Glyphosate	Rainbow trout fry	--	96 hours	LC_{50} = 50 ppm	3 lb a.e./gal.	Folmar 1976
Glyphosate	Bleak	--	96 hours	LC_{50} = 16 ppm	(15-18 ppm) 10°C (50°F)	Linden et al. 1979
Glyphosate, Technical	Rainbow trout	--	96 hours	TL_{50} = 38 ppm	--	U.S. Department of Agriculture 1981
Glyphosate, Technical	Bluegill	--	96 hours	TL_{50} = 78 ppm	--	
Glyphosate, Technical	Bluegill	Dynamic test	96 hours	TL_{50} = 24 ppm	--	
Glyphosate, Technical	Bluegill	--	96 hours	LC_{50} = 120 ppm	--	Monsanto Company 1982a
Glyphosate, Technical	Trout	--	96 hours	LC_{50} = 86 ppm	--	
Glyphosate, Technical	Carp	--	96 hours	LC_{50} = 115 ppm	--	
Glyphosate, Technical	Harlequin fish	--	96 hours	LC_{50} = 168 ppm	--	
Glyphosate, Technical	Carp	Static bioassay	48 hours	TL_{50} = 119 ppm TL_1 = 146 ppm TL_{99} = 96.7 ppm	-- -- --	U.S. Department of Agriculture 1981
		Static bioassay	96 hours	TL_{50} = 115 ppm TL_1 = 125 ppm TL_{99} = 105 ppm	-- -- --	

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments[a]	References
Surfactant used in Roundup®	Rainbow trout	Static bioassay	24 hours 96 hours	LC_{50} = 2.1 ppm LC_{50} = 2.0 ppm	(1.6-2.7 ppm) 12°C (54°F) (1.5-2.7 ppm) 12°C (54°F)	Folmar et al. 1979
Surfactant used in Roundup®	Fathead minnows	Static bioassay	24 hours 96 hours	LC_{50} = 1.4 ppm LC_{50} = 1.0 ppm	(1.2-1.7 ppm) 22°C (72°F) (1.2 [Sic.]-1.7 ppm) 22°C (72°F)	
Surfactant used in Roundup®	Channel catfish	Static bioassay	24 hours 96 hours	LC_{50} = 18 ppm LC_{50} = 13 ppm	(8.5-38 ppm) 22°C (72°F) (10-17 ppm) 22°C (72°F)	
Surfactant used in Roundup®	Bluegill	Static bioassay	24 hours 96 hours	LC_{50} = 3.0 ppm LC_{50} = 3.0 ppm	(2.5-3.7 ppm) 22°C (72°F) (2.5-3.7 ppm) 22°C (72°F)	

[a]Values in parentheses are the 95% confidence limits.

[b]Data not available or not applicable.

Appendix C

Summary of Glyphosate Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	Rererences
Roundup®	Domestic chicken	Eggs immersed in 0, 1, or 5% solution	Immersion for 5 seconds	No significant effects on hatchability or time to hatch over control eggs immersed in 0% solution.	Eggs immersed at 0, 6, 12, or 18 days of embryonic development.	Batt et al. 1980
Glyphosate, Technical	Mallard	Ingestion	8-days	LC_{50} >4,640 ppm	--	Monsanto Company 1982a
Glyphosate, Technical	Bobwhite	Ingestion	8-days	LC_{50} >4,640 ppm	--	Monsanto Company 1982a
Glyphosate	White rock chicken	Injection into egg-10 day old embryos	--	LD_{50} = 12.88 mg/100g egg	Injected into air sac.	Olorunsogo et al. 1978
				LD_{50} = 24.74 mg/100g egg	Injected into yolk sac.	Olorunsogo et al. 1978
Glyphosate	White leghorn chicken	Injection into egg-10 day old embryos	--	LD_{50} = 13.12 mg/100g egg	Injected into air sac.	Olorunsogo et al. 1978
				LD_{50} = 25.44 mg/100g egg	Injected into yolk sac.	Olorunsogo et al. 1978
Glyphosate, Technical	Chickens (adult hens)	Oral, 1,250 mg/kg 2 times daily	Three consecutive days. Repeated for a total dose of 15,000 mg/kg.	No behavioral or microscopic treatment-related changes were observed.	--	Monsanto Company 1982a

Appendix D

Summary of Glyphosate Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Glyphosate	Rat	Ingestion of a single dose	LD_{50} = 4,900 (4,400-5,400) mg/kg	Male and female animals	U.S. Department 1981
			LD_{50} = 4,040 mg/kg		
			LD_{50} = 4,320 mg/kg		Weed Science Society of America 1983
Glyphosate	Rat	Ingestion of a single dose	LD_{50} = 5,600 mg/kg	Summary statement	Monsanto Company 1982a,b
Roundup®	Rat	Ingestion of a single dose	LD_{50} = 5,400 mg/kg	Summary statement	
Rodeo®	Rat	Ingestion of a single dose	LD_{50} >5,000 mg/kg	Summary statement	Monsanto Company 1983b
Glyphosate	Rabbit	Ingestion of a single dose	LD_{50} = 3,800 mg/kg	Summary statement	U.S. Department of Energy 1982
			Acute Dermal Toxicity		
Roundup®	Rabbit	Single application to shaved skin, 24 hr. exposure	Minimum lethal dose (MLD for skin absorption >7,940 mg/kg	Summary statement	Weed Science Society of America 1983
Glyphosate	Rabbit	Single application to shaved skin, 24 hr. exposure	MLD for skin absorption >7,940 mg/kg	Summary statement	
Glyphosate	Rabbit	Single application to shaved skin, 24 hr. exposure	LD_{50} was >5,000 mg/kg	Summary statement	Monsanto Company 1982c
Roundup®	Rabbit	Single application to shaved skin, 24 hr. exposure	LD_{50} was >5,000 mg/kg	Summary statement	
Rodeo®	Rabbit	Single application to shaved skin, 24 hr. exposure	LD_{50} was >5,000 mg/kg	Summary statement	Monsanto Company 1983b

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Skin Irritation		
Rodeo®	Rabbit	Not specified	Practically nonirritating (FHSA score = 0.1/8.0)	Summary Statement	Monsanto Company 1983b
	Rabbit	Repeated dermal contact at concentrations 1 and 5 times 10.8% v/v and 4.0% v/v) the intended use level	Slight skin irritation	Summary statement	Monsanto Company 1983a
Glyphosate	Rabbit	Not specified	Practically nonirritating (FHSA Score = 0.1/8.0)	Summary statement	Monsanto Company 1982a
Roundup®	Rabbit	Not specified	Moderately irritating (FHSA Score = 4.3/8.0)	Summary statement	
Roundup®?	Human	Patch Test, 1 application, dose unspecified	No visible skin changes noted	50 subjects used	U.S. Department of Agriculture 1981
		Patch test, applications 2 through 15, dose unspecified	No visible skin changes noted		
		Sensitization test, dose unspecified	No evidence of injury or sensitization		
			Acute Inhalation Toxicology		
Roundup®	Rats	Four hour exposure to air containing 12.2 ppm Roundup® per liter	No deaths or abnormal reactions	No relevant gross pathology noted when animals sacrificed and autopsied 10 days after exposure	U.S. Department of Energy 1982
Roundup®	Rat	Four hour aerosol exposure	Inhalation LC_{50} was 3.28 ppm	"Slightly toxic"	Monsanto Company 1982b

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Eye Irritation		
Glyphosate	Rabbit	Draize Test	Slightly irritating. (Draize Score = 6.9/110)	Summary statement	Monsanto Company 1982a
Roundup®	Rabbit	Draize Test	Moderately irritating (Draize Score = 18.4/110)	Irritation was completely reversible. All heated eyes returned to normal within 7 days.	
Rodeo®	Rabbit	Draize test	Practically nonirritating (FHSA score = 0.0/110)	--[a]	Monsanto Company 1983b
Roundup®	Rabbit	Undiluted or 5% aqeuous solution	"No more than slightly irritating"	Summary statement	U.S. Department of Agriculture 1981
			Subchronic Oral Toxicity		
Glyphosate	Rat	90-day oral administration; 200, 600 and 2,000 ppm in the diet	No significant abnormalities	Parameters included: body weights, food consumption, behavioral reactions, mortality, hematology, blood chemistry, urinalyses, gross pathology and histopathology	U.S. Department of Agriculture 1981 and Weed Science Society of America 1983
Glyphosate	Dog	90-day oral administration, 200, 600 and 2,000 ppm in the diet	No significant abnormalities	Parameters included were same as for rat. Absolute organ weights, organ to body weight ratios, and organ to brain weight ratios not significantly different in treated and control dogs.	U.S. Department of Agriculture 1981 and Weed Science Society of America 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Dermal Toxicity		
Roundup®	Rabbit	21-day dermal study. Animals treated daily with formulation diluted to its use level or 5 x its use level.	Severe local skin reactions and testicular atrophy	Testicular effects thought to be a manfestation of the stress caused by the skin reaction	U.S. Department of Agriculture 1981
Roundup®	Human	Patches soaked in Roundup® applied to shaved skin for 15 days	No visible skin reaction, no sensitization	--	U.S. Department of Agriculture 1981
			Chronic Toxicity		
Glyphosate	Rat	Groups of 50 males and 50 females fed glyphosate in diet at 0, 30, 100, or 300 ppm for 2 years	No significant differences among control and treated animals, except: (1) percent survival for treatment group did not differ from controls although all values lower than the approximate 50 percent survival expected for this strain of rat; (2) treatment related increase in incidence of cytoplasmic vacuolation and lipid content in liver of animals fed 300 ppm. NOEL = 31 mg/kg/day.	Endpoints included: food consumption, weight gains, final body weights, organ weights, organ to body weight and organ to brain weight ratios; hematologic, clinical chemistry and urine analyses.	Monsanto Company 1983a and U.S. Department of Agriculture 1981
Glyphosate	Dog	Groups of 4 males and 4 females fed glyphosate in diet at 0, 30, 100 or 300 ppm 2 for years	No treatment related abnormalities observed. No evidence of gross or histopathologic changes in liver of dogs	Endpoints included (same as for rat)	U.S. Department of Agriculture 1981
Glyphosate	Mouse	Glyphosate fed in diet at 100 or 300 ppm for 18 months	No evidence of increase in the incidence of cytoplasmic vacuolation or lipid content	--	

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Reproductive and Teratogenicity		
Glyphosate	Rat	Three generation reproduction study. Animals fed diets containing 30, 100 or 300 ppm.	No signficant differences between treatment and control groups regarding viability, weanling weights, litter size, fertility	No adverse effects on parental weights or body weight gains. No deaths attributed to treatment. No gross pathologic difference between controls and treated animals. Organ weights, organ to body weight or brain weight ratios revealed no consistent intergroup differences. No effect on reproductive ability during first generation. Animals fed 300 ppm exhibited reduced mating, fertility, and pregnancy during first litters of second and third generations (F2a and F3a litters). Parameters comparable to controls during the second litters (F2b and F3b) at 30 or 100 ppm no intergroup differences in reproductive parameters. No untoward behavioral reactions or teratogenic effects were observed.	Monsanto Company data cited in U.S. Department of Agriculture 1981
Glyphosate	Rat	Teratology test	No observed teratogenic effects at 3,500 mg/kg/day. Fetotoxicity NOEL = 1,000 mg/kg/day.	Summary statement	U.S. Environmental Protection Agency 1982c
Glyphosate	Rat	Three generation reproduction test	NOEL = 10 mg/kg/day	Summary statement	
Glyphosate	Rabbit	Fetotoxicity test	NOEL = 175 mg/kg/day	Summary statement	

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Reproductive and Teratogenicity (Concluded)		
Glyphosate	Rabbit	Teratogenic study. Pregnant animals treated with 10 or 30 mg/kg body weight during period of fetal organogenesis.	No teratogenic effects observed	Summary statement	U.S. Department of Agriculture 1981
	Rabbit	350 mg/kg/day	No teratogenic effects observed	Summary statement	U.S. Environmental Protection Agency 1982
Roundup®	Deer mouse (Peromyscus maniculatus)	Conifer forest sprayed at 2 lb a.i. per acre	No apparent adverse effects on reproduction, growth, or survival of mice 1 year after treatment	--	Sullivan and Sullivan 1981
			Carcinogenicity		
Glyphosate	Mouse	Animals fed glyphosate in diet at 0, 100 or 300 ppm for 18 months	Gross and microorganic pathologic examinations revealed no correlation between treatment and carcinogenicity	--	Monsanto Company data cited in U.S. Department of Agriculture 1981
Glyphosate	Rat	Groups of 50 male and 50 female animals fed glyphosate in diet at 0, 30, 100 or 300 ppm for 2 years	No gross or histopathologic evidence of any difference in frequency or type of tumors that could be related to treatment	Tissues from 10 male and 10 female rats from control and 300 ppm groups examined. Tissues from smaller numbers of animals from the 30 and 100 ppm groups examined.	U.S. Department of Agriculture 1981

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Concluded)		
Glyphosate	Dog	Groups of 4 male and 4 female beagle dogs fed glyphosate in diet at 0, 30, 100 or 300 ppm for 2 years	No evidence of treatment related induction of tumors presented	--	U.S. Department of Agriculture 1981
Glyphosate	Rat	Glyphosate fed in diet at dosages up to 31 mg/kg body weight/day (equivalent to 600 ppm) for 2 years	No carcinogenic effects reported	Summary statement. Study performed in compliance with FIFRA Guidelines.	Monsanto Company 1983b

[a]Data not available or not applicable.

Appendix E

Summary of Glyphosate Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Mutagenicity		
Glyphosate	Mouse	Dominant lethal test. Males were treated at levels of 5 or 10 mg/kg body weight.	No dominant lethal effect observed. Therefore, non-mutagenic to germ cells.	--[a]	U.S. Department of Agriculture 1981
Glyphosate	Mouse	Dominant lethal test, treatment up to 2,000 mg/kg.	No mutagenic effect.	Summary statement	Monsanto Company 1982a and U.S. Environmental Protection Agency 1982
Glyphosate	*Bacillus subtilis*	Rec assay, tested at concentrations up to 2,000 μg/plate.	No evidence of mutagenicity.	Summary statement	U.S. Environmental Protection Agency 1982
Glyphosate	*Salmonella typhimurium*	Reverse mutation, tested at concentrations up to 2,000 μg/plate.	No evidence of mutagenicity.	Summary statement	
Glyphosate	*S. typhimurium*	Five unspecified strains of *Salmonella* were treated with unspecified concentrations of test material.	No mutagenic activity reported.	Summary statement	Monsanto Company 1982a
Glyphosate	*B. subtilis*	Bacterial cells treated with unspecified concentrations of test material.	No mutagenic activity reported.	Summary statement	Monsanto Company 1982a
Glyphosate	*Saccharomyces cerevisiae*	Yeast cells treated with unspecified concentrations of test material.	No mutagenic activity reported.	Summary statement.	

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Glyphosate

Formulation	Organism	Dose	Comments	References
^{14}C Glyphosate	Rabbits	Single oral dose	Five days after treatment, >80% of ^{14}C were found in feces, 7 to 11% in urine, and <1% in expired $^{14}CO_2$. Most of the remaining $^{14}CO_2$ was found in colon.	U.S. Environmental Protection Agency data as reported in Ghassemi et al. 1981
Glyphosate, Technical ^{14}C material	Bluegills	0.612 ppm	Fish contained a mean tissue ^{14}C-residue concentration of 1.02 mg/kg on day 1, with no further significant increase in ^{14}C-residue content during a 28-day exposure. Mean ^{14}C-residue in edible portion was 1.01 mg/kg (bioconcentration = 1.6x).	U.S. Department of Agriculture 1981
Glyphosate	Channel catfish	10 ppm for 14 days	Maximum glyphosate level never exceeded 0.55 ppm.	Sacher 1978
Glyphosate	Largemouth bass	10 ppm for 14 days	Maximum glyphosate level never exceeded 0.12 ppm.	
Glyphosate	Rainbow trout	10 ppm for 14 days	Maximum glyphosate level never exceeded 0.11 ppm.	
Glyphosate	Rainbow trout	0.02, 0.2, 2.0 ppm	After 12 hours exposure, no residues of either glyphosate or its primary metabolite (aminomethyl phosphonic acid) were detected in fish fillets or eggs from any exposure level.	Folmar et al. 1979
Roundup®	Rainbow trout	0.02, 0.2, 2.0 ppm	In fish exposed to 2.0 mg/kg for 12 hours, fillets had 80 mg/kg of glyphosate and eggs had 60 µg/kg of glyphosate.[a]	

Appendix F (Concluded)

Summary of Metabolism Studies with Glyphosate

Formulation	Organism	Dose	Comments	References
Glyphosate	Cows	--[b]	Milk from cows fed glyphosate had undetectable levels (<0.25 ppm) of glyphosate residues.	Monsanto Company 1982c
Glyphosate	Chickens	--	Bioaccumulation factor in muscle, fat, eggs, and liver as low as 10^{-4}.	Sacher 1978
Glyphosate, Technical ^{14}C material	Bobwhite quail	--	No storage of ^{14}C-labelled material was observed in muscle or fat. Trace residues residues occurred in liver and kidney tissue.	U.S. Department of Agriculture 1981
Glyphosate	Midge larvae	0.02, 0.2, 2.0 ppm	No residues detected.	Folmar et al. 1979
Roundup®	Midge larvae	0.02, 0.2, 2.0 ppm	No residues detected.	
Glyphosate	Chickens, cows and pigs	Up to 75 ppm	Nondetectable (<0.05 ppm) residue in muscle and fat.	Monsanto Company 1982c

[a]Data on fillet tissue residue levels may be in error. See discussion in Section 3.1.2.

[b]Data not available or not applicable.

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Herbicide Background Statement: Hexazinone

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Hexazinone Summary

Major Trade Names: Velpar® and Velpar® Gridball®

Chemical Name: 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione.

Major Forestry Applications: Applied as a selective herbicide for site preparation and for release of conifers and as a nonselective herbicide for weeds and woody plants.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Hexazinone is practically nontoxic to aquatic invertebrates and fish, with LC_{50}'s or no effect levels greater than 10 ppm in invertebrates and greater than 100 ppm in fish. It is relatively nontoxic to birds.

In experimental studies with mammal, hexazinone was mildly toxic by oral, dermal and inhalation routes of administration. Oral intubation of rats, dogs and guinea pigs elicited toxic effects such as body weight loss, salivation, tumors, lethargy, irregular respiration, convulsions, discolored lungs, and heavier livers. Adverse effects on the respiratory and gastrointestinal tracts were also induced by oral inhalation of five hexazinone metabolites. In rabbits and guinea pigs hexazinone was a primary skin irritant, but not a sensitizer. Ocular administration of hexazinone in rabbits resulted in corneal opacity and inhalation or injury of conjunctiva and iris. No treatment-related adverse health effects was observed following inhalation of hexazinone. Subchronic oral studies with hexazinone revealed body weight losses and clinical and histopathological changes related to hepatotoxicity. Dietary administration of hexazinone for 48 weeks to 2 years caused low body weight gains and increased leukocyte and eosinophil counts in rats, nonneoplastic hepatocellular effects in mice, and no adverse effects in hamsters. No reproductive or teratogenic effects was induced by hexazinone treatment in rats or rabbits. Although nonneoplastic effects in liver cells were induced in mice by 2 year dietary hexazinone treatment, no carcinogenic effects were induced in rats or mice. Hexazinone was nonmutagenic in 4 of 5 test systems examined.

Environmental Fate

The primary mechanism for uptake of hexazinone by plants is absorption from soil solution by roots. It is readily metabolized into a variety of metabolites in plants and animals. Hexazinone does not bioaccumulate in animals and both hexozinone and its metabolites are rapidly excreted by animals in urine and feces.

In soil, hexazinone is degraded by light and microbial activity to various metabolites. It is also leached from soil, but tends to leach downward into the soil rather than horizontally. Hexazinone is variably persistent in soil depending on field conditions, with a half-life of one to more than six months. Photodegradation, biodegradation, and dilution are the prime mechanisms for loss of hexazinone activity in aquatic systems. It is moderately persistent in water.

Exposure and Hazard

The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for Forest Service application. Hexazinone is not mutagenic in most test systems and does not appear to be carcinogenic. There should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from hexazinone use if proper case is taken during application.

1.0 General Information

Hexazinone, is also known by trade names* such as Velpar® and Velpar® Gridball®. Gridball® , the pellet form, is no longer manufactured and granules are used in its place. Hexazinone is used to control annual and biennial weeds and woody vines. It is also used to control perennial weeds and grasses in noncropland applications. Although the precise mode of action is as yet unclear, hexazinone is thought to act as a photosynthesis inhibitor. Hexazinone is readily absorbed through both foliage and roots and is translocated, primarily upward, though the plant (Weed Science Society of America 1983).

1.1 Normal Use Patterns

Hexazinone is effective against a wide variety of annual, biennial, and perennial weeds, as well as woody plants. It is used as a nonselective herbicide in noncrop applications and as a selective herbicide for site preparation and for release of conifers and Christmas tree plantations. Hexazinone also has a number of crop uses (blueberries, sugarcane, pineapple, and alfalfa) with established tolerances for each. USDA Forest Service use of hexazinone for 1981 and 1982 is shown in Table 1-1. Hexazinone formulations are listed in Table 1-2.

Application rates for hexazinone depend on a variety of factors, such as size and density of weeds to be controlled and texture and content of organic material in the soil. Registered application rates (U.S. Environmental Protection Agency 1982) range from 0.45 lb a.i. (active ingredient)/acre in sugarcane to 12 lb a.i./acre for noncropland broadcast of liquid formulation. Registered rates for forest use of powder formulations range from 0.5 lb a.i./acre for conifer release to 3 lb a.i./acre (pellets) for conifer site preparation. Hexazinone and all hexazinone-based products were unconditionally registered by the U.S. Environmental Protection Agency on 13 December 1982 in accord with the Federal Insecticide, Fungicide, and Rodenticide Act (Schneider 1984). Registered rates for injection or basal treatment with liquid formulations range from 1 to 2 lb a.i./acre.

1.2 Physical and Chemical Properties

The active ingredient of hexazinone is 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione. It has a

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service Use of Hexazinone

Purpose	Active Ingredient Per Acre (lbs) Range	Average	Acres Treated	Formulation	Application Method
1981					
Aerial					
Site preparation	1.4-2.0	1.6	531	Gridball®	Helicopter
Ground					
Conifer release	1.2	1.2	2,137	Gridball®	Hand Broadcast
General weed control	2.0	2.0	61	Gridball®	Hand Broadcast
Site preparation	1.6-2.0	1.6	1,766	Gridball®	Hand Broadcast
Wildlife habitat improvement	0.8	0.8	36	Gridball®	Hand Broadcast
1982					
Aerial (2 projects)					
Timber	2.0	2.0	3,062	Gridball®	Aerial
Ground (27 projects)					
Timber, Nursery	0.5-4.0	2.2	6,962[a]	Liquid	Ground Foliar
Timber, Wildlife, Range	0.2-2.0	1.2		Gridball®	Hand Broadcast

[a]Acres treated with liquid and Gridball® combined.

Source: Gross 1983.

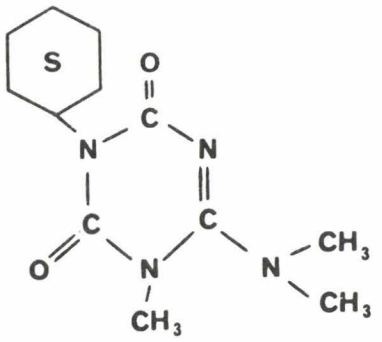
Table 1-2

Hexazinone Formulations

Formulation	Active Ingredient
Velpar® Weed Killer	90% hexazinone, soluble powder
Velpar® Gridball® Brush killer	10% or 20% hexazinone in pellet form
Velpar® L Weed Killer	25% hexazinone (240 g/ , 2 lb/gal.) miscible liquid
BO-RID V-4 Liquid Weed killer	0.5% hexazinone, liquid
Mobley V-4 Liquid Weed killer	0.48% hexazinone, liquid
Pronone 10G	10% hexazinone in granular form

Source: U.S. Environmental Protection Agency 1982; Weed Science Society of America 1983; and Gross 1983.

molecular formula of $C_{12}H_{20}N_4O_2$ and a molecular weight of 252.3. The structural formula is given below (Weed Science Society of America 1983).



In its pure form, hexazinone is a white crystalline solid with a negligible odor. It is heavier than water having a specific gravity of 1.25, melts at 239° to 243°F (115° to 117°C), and has a relatively low vapor pressure (6.4×10^{-5} mm Hg at 187°F [86°C] which extrapolates to 2×10^{-7} mm Hg at 77°F [25°C]). At 77°F (25°C), the water solubility of hexazinone is 3.3 percent (3.3 grams per 100 grams water). It is much more soluble in most organic solvents than it is in water, with solubilities of 388 grams/100 grams chloroform, 265 grams/100 grams methanol, 94 grams/100 grams benzene, 83.6 grams/100 grams dimethylformamide, 79.2 grams/100 grams acetone, 38.6 grams/100 grams poluene, and 0.3 grams/100 grams hexane. Hexazinone is relatively stable under normal conditions with a shelf-life of at least 2 years (Weed Science Society of America 1983 and DuPont undated).

2.0 Toxicity of Hexazinone

Very little published data are currently available relating to the toxicological properties of hexazinone. Most of the data constitute proprietary information that have been obtained for registration and use permits. Available data on the toxicological properties of hexazinone are summarized in Table 2-1 and show that this herbicide has a low toxicity in most organisms tested. Hexazinone metabolites have been shown to have a low toxicity to animals as tested. Studies with rodents indicate that hexazinone is noncarcinogenic, nonteratogenic, and has little or no effect on fertility, reproduction or development. Hexazinone is not mutagenic in most test systems.

2.1 Invertebrates and Microorganisms

Hexazinone formulated as soluble powder, pellets, dry flowable and liquid end-use products is practically nontoxic to aquatic invertebrates (U.S. Environmental Protection Agency 1982). The studies of hexazinone toxicity with invertebrates and microorganisms that are summarized in Appendix A indicate that hexazinone has a low toxicity, with LC_{50}'s or no effect levels above 10 ppm. An exception is algae, in which population growth is inhibited at levels as low as 0.5 ppm hexazinone.

Most toxicity studies with invertebrates and microorganisms have been done in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of hexazinone in a natural environment. For example, in the laboratory, aquatic organisms were generally kept up to 4 days or more in static conditions (in tanks or aquaria without a flow-through of freshwater). Microorganisms were grown in culture media in the laboratory. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to hexazinone or other man-made chemicals is usually a transient phenomenon whose duration depends on the rate of streamflow or on the rate of decomposition of the chemical. Additionally, in natural systems, interactions with other man-made and naturally occurring chemicals, and changes in environmental factors, may affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions could alter the tolerance levels of organisms to the herbicides.

The toxic effects of hexazinone in insects have been illustrated by only one reported study with honeybees. Topical application at a rate of 60 μg hexazinone per bee resulted in 10 percent mortality during a 48-hour test. The LC_{50} was estimated to be >60 μg per bee (Schneider 1984).

Table 2-1

Summary of Toxicological Properties of Hexazinone[a]

1. Acute Oral Toxicity

Organism (Formulation)	Adverse Effects
Rat (0.5 and 25% liquid hexazinone, 10 and 20% Gridball®, 66% wettable powder, and 89.3% active ingredient)	LD_{50}> 4,495 mg/kg
Rat (Hexazinone, unformulated)	LD_{50} = 1,690 to 2,012 mg/kg
Rat (Hexazinone in corn oil)	ALD[b] = 1,500 to 5,000 mg/kg
Guinea pig (Hexazinone, unformulated)	ALD = 860 mg/kg
Beagle Dog (Hexazinone, (unformulated)	ALD > 3,400 mg/kg
Rat (Metabolites A-E)	ALD > 4,686 mg/kg
Fish (various formulations)	LC_{50}> 100 ppm
Birds (Hexazinone)	LD_{50} = 2,258 mg/kg

2. Acute Dermal Toxicity

Organism (Formulation)	Adverse Effects
Rabbit (Hexazinone, 90% soluble powder, 60 and 66% dry flowable, and unformulated.)	LD_{50} > 2,000 mg/kg Not a primary skin irritant. Mild erythema reverted to normal.
Rabbit (Hexazinone, 0.5% and 25% liquid)	LD_{50} > 2,000 mg/kg
Guinea pig (Hexazinone, unformulated)	Not a skin irritant or sensitizer.
Guinea pig (Hexazinone, 25% liquid)	Mild transient irritation. Not a sensitizer.

Table 2-1 (Continued)

3. Eye Irritation (Concluded)

Organism (Formulation)	Adverse Effects
Rabbit (Hexazinone, unformulated and 90% soluble powder, 89.3% active ingredient)	Mild-to-moderate corneal opacity and moderate-to-severe conjunctivitis and mild-to-moderate iritis that reverted to normal in 7 to 21 days in washed eyes. Corneal injury persisted to 28 days in unwashed eyes.
Rabbit (Hexazinone, 66% wettable powder)	Eye irritant. Corneal opacity persisted to day 14 in 2 of 6 rabbits.
Rabbit (Hexazinone, 0.5, 1.25, and 5.0% liquid)	Either no eye irritation or mild corneal opacity. Moderate iritis and moderate conjunctivitis reverted to normal in washed and unwashed eyes.
Rabbit (Hexazinone, 25% liquid)	Eye irritant. Mild-to-severe corneal opacity, mild-to-moderate iritis and moderate-to-severe conjunctivitis reverted to normal or slight corneal and conjunctival damage after 21 to 35 days in washed and unwashed eyes.

4. Inhalation Toxicity

Organism (Formulation)	Adverse Effects
Rat (Hexazinone, unformulated and 90% soluble or wettable powder and 0.5% liquid)	ALD $>$ 7.3 ppm. No deaths at 7.3 ppm and below. Irregular respiration and salivation during exposure. No adverse clinical or pathological findings following single and repetitive exposure.

Table 2-1 (Continued)

5. Subchronic Oral Toxicity

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rat (Hexazinone, unformulated)	2 weeks	300 mg/kg/day by intubation 5 days per week	No adverse toxic effects.
Rat (Hexazinone, unformulated)	90 days	0 to 5,000 ppm in diet	Decreased body weight and food efficiency at 5,000 ppm.
Rat (Hexazinone, unformulated)	2 weeks	1,000 mg/kg/day by intubation 5 days per week.	Increased lion weight and larger hepatocytes. Wet and stained perinea, congestion, slight weight loss, and salivation.
Mouse (Hexazinone, unformulated)	8 weeks	0 to 10,000 ppm in diet	Increased liver weight at 10,000 ppm.
Hamster (Hexazinone, unformulated)	8 weeks	0 to 10,000 ppm in diet	No adverse toxic effects.
Dog (unformulated)	90 days	0 to 5,000 ppm in diet	NOEL = 1,000 ppm. Decreased body weight gain, food consumption and albumin: globulin ratio and increased alkaline phosphatase and liver weight at 5,000 ppm.
Cottonail rabbits, prairie voles, white-tailed deer, Norway rats, grey squirrels, racoons, skunk, and opossum (Gridball®)	3 to 5 weeks	2 to 100 pellets in feeding area	No adverse toxic effects.

Table 2-1 (Continued)

6. Acute Intraperitoneal Toxicity

Organism (Formulation)	Adverse Effects
Rat (Hexazinone, unformulated)	LD_{50} = 530 mg/kg body weight; weight loss, pallor, salivation, nose and mouth bleeding, labored irregular respiration, lethargy, prostration, ruffled fur, and colonic convulsions.

7. Teratology, Reproduction and Fertility

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Hexazinone, 90% wettable powder)	0 to 2,500 ppm in diet for 3 generations, 3 litter study.	No adverse effects on reproduction or lactation. Body weights in 2,500 ppm group F_2 and F_3 pups lower than in untreated control pups at weaning.
Rat (Hexazinone, unformulated)	0 to 5,000 ppm in diet from day 6 through 19 of gestation.	No evidence of embryotoxicity, teratogenicity or clinical signs of toxicity except slightly reduced maternal body weight gain and food consumption.
Rabbit (Hexazinone unformulated)	0 to 125 ppm by intubation from day 6 through 19 of gestation.	No embryo-fetal toxicity or teratogenicity.

8. Carcinogenicity

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Hexazinone, 90% soluble powder)	0 to 10,000 ppm in diet for 2 years	NOEL = 200 ppm. No carcinogenic effects.
Mouse (Hexazinone unformulated)	0 to 10,000 ppm in diet for 2 years	NOEL = 200 ppm. No carcinogenic effects.

Table 2-1 (Concluded)

9. Mutagenicity

Organism (Formulation)	Adverse Effects
Salmonella typhimurium 5 strains, unspecified	Nonmutagenic.
Chinese Hamster Ovary Cell Point Mutation Assay	Nonmutagenic.
Chinese Hamster Ovary Cell *in vitro* Cytogenetic Assay	Chromosome damage induced with and without activation conditions.
Rat *in vivo* Bone Marrow Cytogenetic Assay	No chromosome aberrations detected.
Rat Hepatocyte *in vitro* unscheduled DNA synthesis Assay	No induction of unscheduled DNA synthesis.

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.
[b]Average Lethal Dose.

Crustacea showed a wide variation in response to hexazinone depending upon both the species tested and the experimental design. In crustacea, the reported range of toxic effects for hexazinone varies from LC_{50} 20 to 50 ppm during 21-day life cycle studies with Daphnia, a freshwater crustacean, to a 96-hour LC_{50} greater than 1,000 ppm in the fiddler crab, an intertidal species (U.S. Environmental Protection Agency 1982).

A field study describing aquatic and terrestrial invertebrate responses to hexazinone pellets applied to a forested watershed at a rate of 15 lb/acre is reported by Mayack et al. (1982). Over a period of 8 months following hexazinone application, there were no major alterations in aquatic invertebrate species composition or diversity and no changes in terrestrial invertebrate (microarthropod) community compositions.

Rhodes et al. (1980) reported that 10 ppm hexazinone incorporated into laboratory soil cultures did not reduce population counts of either fungi or bacteria during an 8-week exposure period. They also reported that, during a 5-week exposure period, 5 and 20 ppm hexazinone incorporated into laboratory soil cultures did not adversely affect the bacterial soil nitrification process.

2.2 Fish

Available studies of the toxicity of hexazinone to fish, summarized in Appendix B, indicate that this herbicide is only slightly toxic. The LC_{50}'s were greater than 100 ppm in all studies reported. Clarke et al. (1970) propose that chemicals with fish LC_{50}'s greater than 10 ppm be considered "slightly toxic." The U.S. Environmental Protection Agency (1982) describes technical hexazinone as "practically nontoxic" to warmwater and coldwater fish.

2.3 Birds

The toxic effects of hexazinone in birds have been investigated in studies that are summarized in Appendix C. In general, hexazinone is relatively nontoxic to bird species tested. The acute oral LD_{50} for bobwhite quail is reported to be 2,258 mg hexazinone per kg (U.S. Environmental Protection Agency 1982). The LC_{50} was greater than 10,000 ppm in young bobwhite quail and mallards fed varying amounts of hexazinone in their diet for 5 days of an 8-day study (DuPont data cited in Ghassemi et al. 1981 and 1982).

Birds do not seem to be attracted to hexazinone, as evidenced by a study in which Velpar® Gridballs® were placed near the food of captive black-capped chickadees and ring-necked pheasants, as well as near feeding sites frequented by these 2 species and by 5 other bird

species. During an observation period of up to 3 weeks, birds made no response to the Gridballs® (Richmond 1979).

2.4 Mammals

The potential health hazards from acute and chronic exposure to hexazinone have been evaluated in laboratory animals such as rats, mice, guinea pigs, rabbits, dogs, and hamsters. A few observations of common wildlife mammals have been reported. The results of the toxicity tests summarized in Table 2-1, Appendix D, and Appendix E are discussed below.

Hexazinone is considered to be only slightly toxic (DuPont undated and Ghassemi et al. 1981). On the basis of the animal studies reported to date, there is no concern for carcinogenic or teratogenic hazards with hexazinone. Hexazinone is nonmutagenic in 4 of 5 test systems examined.

2.4.1 Acute Toxicity

Acute toxicity data for hexazinone are reported in Table 2-1 and Appendix D. By oral, dermal, and inhalation routes, hexazinone formulations are mildy toxic. In eye irritation studies, hexazinone formulations were moderately toxic causing damage to the cornea, conjunctiva, and iris. Most eye irritation was reversible.

2.4.1.1 Oral. As summarized in Table 2-1 and Appendix D, single doses of hexazinone administered in substantial amounts by intubation induced mild toxic effects over a 14-day observation period in rats, dogs, and guinea pigs. Intubation of single doses of unformulated hexazinone yielded LD_{50}'s of 1,690 to 2,012 mg/kg body weight in rats, an ALD greater than 3,400 mg/kg body weight in beagle dogs, and an LD_{50} equal to 860 mg/kg body weight in guinea pigs. All LD_{50}'s are in the U.S. Environmental Protection Agency's toxicity category III, warranting the handling of hexazinone with caution. The most common toxic effect observed at lethal or near lethal doses were body weight loss, salivation, tremors, lethargy, irregular respiration, prostration, congestion, and convulsions.

In rats, administration of 300 mg/kg/day of hexazinone by intubation suspended in corn oil was repeated five times a week for two weeks. All rats survived with no clinical evidence of toxicity and no apparent treatment-related histopathology (Schneider and Kaplan 1983). Adverse effects induced by formulated hexazinone materials at lethal or near lethal doses also occasionally included chromodacryorrhea (colored eye fluid), discolored lungs, slightly heavy liver, and enlarged hepatocytes. The formulated materials were 0.5 and 25 percent liquid hexazinone, 10 and 20 percent

Gridball®, 66 percent wettable powder and 89.3 percent "active ingredient." In general, the formulated materials were less toxic than unformulated hexazinone (see Appendix D).

Toxicological Properties of Hexazinone Metabolites

The acute oral toxicities of 5 metabolites (Metabolites A-E) of hexazinone have been tested and found to be very slightly toxic. All of the ALD's were greater than 4,686 mg/kg body weight. The chemical names, ALD's, and structures of all five metabolites are detailed in Appendix D-1. The acute oral toxicities for all of the metabolities were evaluated in male Crl:CD rats using the same experimental protocol. Single doses of corn oil suspensions of each metabolite were administered by intubation to one animal per dose level. The dose levels were not specified. The animals were weighed and observed for 14 days. The ALD's for each of the metabolites were as follows: ALD > 4,686 mg/kg for Metabolite A, ALD > 5,000 mg/kg for Metabolite B, ALD = 7,500 mg/kg for Metabolite C, ALD > 7,500 mg/kg for Metabolite D, and ALD = 7,500 mg/kg for Metabolite E. Weight loss and wet or stained perinea were observed after dosing with all of the metabolites. Diarrhea occurred after Metabolite A, D, and E dosing. Respiratory abnormalities were induced by Metabolites C, D, and E. Chromodacryorrhea was reported following treatment with Metabolites D and E (Schneider and Kaplan 1983).

2.4.1.2 Acute Intraperitoneal Toxicity. Mild toxicity was observed during a 14-day observation period following single intraperitoneal injections of unformulated hexazinone in rats. The LD_{50} was 530 mg/kg with 95 percent confidence limits between 380 and 570 mg/kg body weight. The adverse effects observed in this study and listed in Table 2-1 include weight loss, pallor, salivation, nose and mouth bleeding, irregular respiration, lethargy, prostration, ruffled fur, and convulsions (Schneider and Kaplan 1983).

2.4.1.3 Dermal. Hexazinone is not considered to be a skin irritant or sensitizer in rabbit or guinea pigs (Table 2-1). The general test protocol used was to apply the test material to intact or abraded shaved areas of skin, to cover the area with gauze and occlusive wrappings, and then to make observations after 1 to 14 days. Variations of this protocol are indicated for 13 dermal studies described in Appendix D. An LD_{50} greater than 2,000 mg/kg body weight in rabbits was reported for unformulated hexazinone, 0.5 and 25 percent liquid, 90 percent soluble powder, and 60 and 66 percent dry flowable hexazinone. In rabbits and guinea pigs mild redness or inflammation of the skin that reverted to normal was occasionally noted. The only adverse effect noted was an elevated enzyme (glutamic-pyruvic transaminase) activity, with no histopathologic evidence of liver

injury, in rabbits treated with a 90 percent soluble powder formulation of hexazinone (Ghassemi et al. 1981 and Schneider and Kaplan 1983). The formulations were not considered to be primary skin irritants. Additional treatment of guinea pigs to determine skin sensitization involved a follow-up series of intradermal injections, followed by skin applications and an extended observation period (Appendix D). No sensitization was observed.

2.4.1.4 Eye Irritation. Formulated and unformulated hexazinone induced eye irritation in rabbits. Corneal opacity or inflammation of the conjunctiva and iris persisted 7 days or longer in some animals. The general format for the 15 eye irritation studies described in Appendix D follows. The eyes were treated with the test material. Some eyes were washed. Both washed and unwashed eyes were examined for intervals between one hour and 35 days following treatment. The liquid and most concentrated hexazinone formulations induced the most severe and persistant eye irritation. These formulations were 25 percent hexazinone liquid, 90 percent soluble powder, 89.3 percent active ingredient and unformulated hexazinone. The ranges of adverse ocular effects included mild to moderate corneal opacity, moderate to severe conjunctivitis, and mild to moderate inflammation of the iris. All conditions reverted to normal within 7 to 21 days in washed eyes. The 66 percent wettable powder formulation induced corneal opacity that persisted to day 14 in 2 of 6 rabbits. The less concentrated formulation, 25 percent liquid hexazinone, induced injury to cornea, conjunctiva, and iris. Corneal opacity, cloudiness, or irritation and conjunctivitis occasionally persisted through 21 to 35 days. The most dilute formulations tested were 0.5, 1.25, and 5.0 percent liquid hexazinone. All eye irritation induced by the 5 percent formulation reverted to normal within 14 days (Table 2-1). No ocular irritation was produced by then 1.25 and 0.5 percent formulations.

2.4.1.5 Inhalation. Hexazinone is mildly toxic (ALD >7.3 ppm) to rats by the inhalation route (Table 2-1 and Appendix D). No deaths resulted from single or repetitive whole body or head-only exposures to doses from 0 to 7.48 ppm. No clinical or pathological abnormalities were observed 14 days after exposure. One study reported the occurrence of irregular respiration and salivation during exposure (Ghassemi et al. 1981). Otherwise, no behavioral or treatment-related abnormalities developed in response to unformulated, or wettable powder forms of hexazinone.

2.4.2 Subchronic Toxicity

Subchronic oral toxicity tests with unformulated hexazinone and Velpar® in laboratory animals and with Gridball® in wildlife animals

yielded few adverse effects (Table 2-1). The 90-day NOELs were 1,000 ppm in rats and dogs and the 8-week NOEL was 5,000 ppm in mice (U.S. Environmental Protection Agency 1982 and Ghassemi et al. 1981). Male rats intubated 5 days/week for 2 weeks with 300 mg/kg hexazinone exhibited no adverse effects and 100 percent survival. Male rats intubated with 1,000 mg/kg 5 days/week for 2 weeks developed increased liver weights and larger hepatocytes and congestion, slight weight loss, salivation and wet and stained perinea (Schneider and Kaplan 1983). Thirty-day old male and female rats were provided 0 to 5,000 ppm hexazinone in the diet for 90 days (Ghassemi et al. 1981 and Newton and Dost 1981). Decreased body weight and food efficiency were observed at 5,000 ppm. None of the treatment groups showed other clinical, nutritional, hematological, urinary, or biochemical evidence of toxicity (U.S. Environmental Protection Agency 1982). Histopathological examination of tissues from the 5,000 ppm treatment group revealed no toxic effects.

Laboratory mice of both sexes fed hexazinone at concentrations of 0 to 10,000 ppm for 8 weeks showed no mortality or clinical and pathological signs of toxicity. Only increased liver weight in 10,000 ppm treated male and female mice was observed (Ghassemi et al. 1981 and Newton and Dost 1981). Hamsters fed 0 to 10,000 ppm hexazinone for 8 weeks developed no clinical signs of toxicity (Ghassemi et al. 1981). For beagle dogs fed 0 to 5,000 ppm unformulated hexazinone for 90 days, the NOEL was 1,000 ppm. At 5,000 ppm, decreased body weight gain, elevated alkaline phosphatase, lowered albumin:globulin ratios, and increased liver weight resulted. No histopathological changes in liver were observed (U.S. Environmental Protection Agency 1982; Ghassemi et al. 1981; and Newton and Dost 1981).

The results of placing Velpar® Gridball® pellets in the feeding area of common wildlife mammals are summarized in Table 2-1. Cottontail rabbits, prairie voles, white-tailed deer, Norway rats, and grey squirrels were exposed to Gridball® pellets in their natural habitat. Raccoons, skunks, and an opossum were live-trapped and exposed to Gridball® pellets. Among all of these animals, even when deprived of regular diet, only one prairie vole nibbled the Gridball® pellets. No mortality, unusual behavior, or apparent illness were observed in these wildlife mammals (Richmond 1979).

2.4.3 Chronic Toxicity

No behavioral, clinical or pathological signs of toxicity were observed following 48 weeks to 2 years of dietary hexazinone administration to rats, mice, and hamsters (Table 2-1). Continuous feeding of 0 to 2,500 ppm 90 percent wettable powder hexazinone for 2 years

yielded a NOEL of 200 ppm in rats. Clinical pathology was evaluated at intervals from 1 to 24 months. Lower body weight gains, lower food efficiency and food consumption, and increased leukocyte and eosinophil counts were observed in the 2,500 ppm male rats. Decreased body weight gains were also observed in the female rats fed 1,000 ppm (U.S. Environmental Protection Agency 1982). No gross or histopathological changes and no carcinogenicity could be attributed to hexazinone treatment (Ghassemi et al. 1981; U.S. Environmental Protection Agency 1982; and Newton and Dost 1981).

In a second chronic study, mice were fed 0 to 10,000 ppm unformulated hexazinone for 2 years. Hematology was conducted at six intervals from 1 to 24 months. Nonneoplastic hepatocellular effects were noted in males and females at 1,000 ppm and in males at 2,500 ppm. The NOEL was 200 ppm. No behavioral, clinical, or nutritional signs of toxicity were reported and no carcinogenic effects were observed (Schneider and Kaplan 1983).

The third chronic toxicity study tested the effects of unformulated hexazinone in male and female hamsters which were fed 5,000 ppm hexazinone in the diet. At 48 weeks, this study was interrupted due to an intercurrent infectious disease. No adverse effects related to test material administration were reported (Newton and Dost 1981).

2.4.4 Reproductive and Teratogenic Effects

No adverse reproductive or teratogenic effects of hexazinone in rats or rabbits are reported in three studies that are summarized in Table 2-1. In a three generation, three litter reproduction study, male and female rats were fed 0 to 2,500 ppm hexazinone through maturation, reproduction, and weaning of an F_3 litter. The 90 percent wettable powder hexazinone had no adverse effect on reproduction, or lactation. Lower weanling weights of the 2,500 ppm group F_2 and F_3 litters compared with the untreated control group were reported (Newton and Dost 1981). The NOEL was 1,000 ppm.

In a teratology study, pregnant rats were fed 0 to 5,000 ppm unformulated hexazinone from day 6 through day 15 of gestation. All animals were sacrificed on day 21 of gestation. A slight depression in maternal body weights occurred at 1,000 and 5,000 ppm. Only at 5,000 ppm were there slightly lower food consumption and body weight gain of pregnant females. No evidence of embryotoxicity or teratogenicity resulted (U.S. Environmental Protection Agency 1982 and 1983; Ghassemi et al. 1981; and Newton and Dost 1981). Hexazinone caused no adverse effects on the number of implantations, resorptions, live fetuses per litter, and mean weight and crown-rump length of the fetuses. No malformations or major abnormalities were observed in fetuses exposed to the test material.

A second teratology study was carried out in groups of female rabbits artifically inseminated and later dosed with 0 and 20 to 125 mg/kg/day of unformulated hexazinone on days 6 through 19 of gestation (U.S. Environmental Protection Agency 1982). The test dams had only minor differences in clinical signs and body weight from controls and no gross pathological lesions. No differences were reported between controls and test animals in pregnancy rate, uterine weight, corpora lutea, implantations, and fetal viability or size. Notable soft tissue and skeletal abnormalities that were not dose dependent were observed in four pups. Variants in skeletal development including delayed ossification in extremities and extra ribs were higher at 125 mg/kg (16.2 percent) than in the control (5.3 percent). The NOEL was 125 mg/kg for embryo-fetal toxicity and teratogenicity.

2.4.5 Carcinogenicity

As illustrated in two studies in Table 2-1, no carcinogenic effects were induced by feeding hexazinone to rats and mice for 2 years. Male and female rats were fed 0 to 2,500 ppm 90 percent wettable powder formulation of hexazinone. The diets were initiated with weanlings and continued for 2 years. No carcinogenic or other toxic effects were reported (Ghassemi et al. 1981 and Newton and Dost 1981).

In the second study, hexazinone was fed in doses of 0 to 10,000 ppm to mice for 2 years. There was no evidence for compound-related carcinogenicity; however, nonneoplastic effects in liver cells were observed in both sexes at 10,000 ppm and in males at 2,500 ppm.

2.5 Mutagenicity

Table 2-1 summarizes the mutagenic potential of unformulated hexazinone. The test material was negative in four of the five systems used. Hexazinone is nonmutagenic in the Ames bacterial assay. Five strains of Salmonella typhimurium gave negative results with unformulated hexazinone in the absence or presence of liver microsomal activation (U.S. Environmental Protection Agency 1983; Newton and Dost 1981; and Ghassemi et al. 1981). The concentrations tested ranged from 400 to 2,000 μg/plate.

Two test systems used Chinese Hamster Ovary (CHO) cells. Hexazinone was nonmutagenic in the CHO cell point mutation assay. For this test, CHO cells were incubated in medium containing hexazinone with and without an activation system. The cells were subcultured to allow growth and expression of cytotoxicity, then seeded in medium with and without selective agents to allow enumeration of HGPRT deficient mutants.

In the CHO in vitro cytogenic assay, unformulated hexazinone gave positive results. CHO cells were exposed to hexazinone concentrations of 2, 4 and 5 mg/ml in the presence and absence of an activation system. The cells were arrested in metaphase with colcemid then scored for chromosome aberrations. Chromosome damage (clastogenicity) was induced under nonactivation conditions at hexazinone concentrations of 4 and 5 mg/ml but not 2 mg/ml. Chromosome damage was also induced under activation conditions in one of two assays with 4 mg/ml hexazinone (Schneider and Kaplan 1983).

The fourth test system was an in vivo bone marrow cytogenetic assay using Sprague-Dawley rats. Single doses of 100 to 1,000 mg hexazinone/kg body weight were administered to groups of 12 rats per sex. Colchicine was administered to 3 rats per sex per group at 6, 12, 24 and 48 hours after hexazinone to arrest the cells in metaphase. The rats were sacrificed. Bone marrow cells were collected from the femurs, prepared, and examined. No hexazinone-related chromosomal aberrations were detected. Five deaths occurred in the 1,000 mg/kg group. Toxic signs observed were depression at 300 mg/kg and convulsions at 1,000 mg/kg (Schneider and Kaplan 1983).

The fifth in vitro test system was to evaluate the effect of hexazinone on unscheduled DNA synthesis (Appendix E). Freshly isolated hepatocytes were treated with unformulated hexazinone followed by radio-labeled thymidine (Schneider and Kaplan 1983). Thymidine incorporation into DNA was quantitated autoradiographically. No induction of unscheduled DNA synthesis was observed.

3.0 Environmental Fate

Hexazinone is effective against a wide variety of annual, biennial, and perennial weeds and woody plants. It is variably persistent in soils, with a half-life of one month to more than six months, depending upon specific field conditions; is moderately persistent in water; and bioaccumulates only slightly in animals. Hexazinone is absorbed primarily via plant roots from which it is translocated to plant foliage (U.S. Environmental Protection Agency 1982).

3.1 Bioaccumulation and Metabolism

Studies of hexazinone metabolism in plants and animals have demonstrated eight metabolites of hexazinone involving loss of methyl groups (CH_3) or aminomethyl groups ($NHCH_3$), ring oxidation, or addition of hydroxyl groups (OH) to the parent molecule. The structures of five metabolites for which toxicity data are reported are illustrated in Appendix D-1. Some of these metabolites have been identified as a bound form to normal metabolic products or intermediates (Holt 1981; Rhodes 1980a and 1980b; and Rhodes and Jewell 1980). Toxicity studies with these five hexazinone metabolites are reviewed in Section 2.4.1 and Appendix D-1.

3.1.1 Plants

The primary mechanism for uptake of hexazinone by plants is absorption from soil solution by roots. The utility of Velpar® Gridball® pellet application takes advantage of this mechanism. The pellets are broken down by rainfall and leached through the soil to the root zone in a concentrated column. As a supplement to root absorption, liquid formulations applied as a foliar spray may be directly absorbed through the foliage (Ghassemi et al. 1982).

In a metabolism study with greenhouse-grown sugarcane, plant tissues were sampled 6 months after application of ^{14}C-labeled hexazinone at rates equivalent to 0.45 and 0.89 lb a.i./acre (0.5 and 1.0 kg/ha). Plant tissue residues of hexazinone and its metabolites were 0.05 ppm and 0.07 to 0.08 ppm in the sugarcane treated at 0.45 and 0.89 lb a.i./acre respectively. These tissues consisted primarily of hexazinone metabolites with less than 1 percent parent hexazinone (DuPont data cited in U.S. Environmental Protection Agency 1982).

In a field study, a 12 x 12 foot plot of alfalfa was treated with ^{14}C-hexazinone at a rate of 1 lb a.i./100 gal./acre (1.12 kg/ha). Three crops of alfalfa, harvested 2, 3 and 6 months following herbicide application, contained ^{14}C-residues calculated as 0.6, 0.5, and 0.1 ppm, respectively, as hexazinone. Of the total radioactivity

recovered in these samples, most was identified as belonging to natural plant products. (DuPont data cited in U.S. Environmental Protection Agency 1982). In this study, the first harvest contained only 3 percent hexazinone and approximately 12 percent of free and conjugated metabolites (A, B, and C). No intact hexazinone or metabolities are found in the second or third harvests (Schneider 1984).

The U.S. Environmental Protection Agency (1982) also reports DuPont data on crop residues in alfalfa hay and alfalfa forage and in sugarcane obtained from studies conducted in a wide range of geographical areas. Alfalfa forage and hay were treated with 0.5 to 1.5 lb a.i. hexazinone per acre (0.56 to 1.68 kg/ha). Maximum residues of 0.88 ppm hexazinone and 0.54 ppm total metabolites (assuming measurements below detectable limits equal 0.1 ppm) were detected in green forage. Maximum residues of less than 0.04 ppm hexazinone and 1.22 ppm total metabolites were detected in alfalfa hay. Sugarcane was treated twice with 2 lb hexazinone a.i./acre/application (2.24 kg/ha). Less than 0.14 ppm total of hexazinone plus metabolites was found in raw cane or juices and less than 0.3 ppm total of hexazinone plus metabolites was found in molasses.

In another series of studies, when sugarcane was treated with 5.4 and 7.2 lbs hexazinone a.i./acre (6.05 and 8.06 kg/ha), less than 0.04 ppm hexazinone or metabolites was detected in the cane, juice, molasses, and sugar (DuPont data in U.S. Environmental Protection Agency 1982).

Hawaiian pineapple plants were spray treated with ^{14}C-hexazinone at 2 lb ai/acre and kept in a greenhouse for 7 months until harvest of mature pineapples. ^{14}C-hexazinone was rapidly metabolized in the plants. Total ^{14}C in pineapple pulp was approximately 0.4 ppm. While no intact ^{14}C-hexazinone (< 0.01 ppm) was detected, metabolites A, B, C, D, and F, which were partly conjugated, were observed. Radioactive polar material present in pineapple pulp (20-24 percent of the total ^{14}C) represented incorporation of ^{14}C-hexazinone breakdown products into natural products, mostly sugars. The half-life of ^{14}C-hexazinone in pineapple leaves and soil was 3 and 4 months, respectively, with the same metabolites being formed (Schneider 1984).

Alfalfa, sorghum, and onions were grown in a greenhouse on Keyport silt loam which had been treated with ^{14}C-hexazinone at 1.5 lb/acre (aged 4 and 12 months) and 4 lb/acre (aged 12 months). Total radioactivity in the aged soils at planting was 0.20, 0.06, and 0.10 respectively, calculated as ^{14}C-hexazinone. Intact hexazinone was present only in trace levels (≤ 0.01 ppm). Several days after seedling emergence, alfalfa showed some reduced stand (about 50

percent) in soils containing 0.1-0.2 ppm ^{14}C, and onion seedlings in soil containing 0.2 ppm total ^{14}C also had reduced survival (about 25 percent less than control). However, both alfalfa and onion seedlings which survived the first week grew normally and did not show any indication of injury. No reduced stand was observed with sorghum. Total ^{14}C in alfalfa at harvest was 0.2-0.3 ppm, in the sorghum plant 0.1-0.4 ppm, in sorghum grain 0.01-0.05 ppm, and in onions 0.01-0.02 ppm. An average of 89 percent of the total ^{14}C in alfalfa, 64 percent in sorghum plant, and 40 percent in onions was found in the bound form, likely incorporated into natural products, as demonstrated previously in alfalfa metabolism studies (Schneider 1984).

3.1.2 Animals

Animal metabolism studies with hexazinone are summarized in Appendix F and show that hexazinone is readily metabolized in animals. Ingested hexazinone is rapidly excreted in urine and eliminated in feces. It does not bioaccumulate appreciably in animals, and tissue residues of both hexazinone and its metabolites decrease rapidly when exposure to hexazinone ceases.

Several rat metabolism studies with radiolabeled hexazinone indicated that most of the material and its metabolites are eliminated within the first 24 hours, with essentially complete elimination within three to four days of dosing. Animals pretreated with hexazinone clear themselves of hexazinone more rapidly than do animals that have not been pretreated, indicating that pretreatment results in metabolic adaptation and more rapid excretion (Rhodes and Jewell 1980 and Schneider and Kaplan 1983). When female goats were fed 1 to 5 ppm hexazinone in their diet daily for five days, 0.02 to 0.05 ppm hexazinone residues were detected in milk for the 1 and 5 ppm doses, respectively by the second day. No residues of hexazinone or of metabolites A, D, or E and only trace levels (maximum 0.05 ppm) of metabolite B were found in cow's milk from animals fed up to 25 ppm in their diet for 30 days (Schneider and Kaplan 1983). Bluegill sunfish, exposed for 4 weeks to up to 1 ppm hexazinone, had maximum residues of 2.1 ppm in the carcass and 6.7 ppm in viscera. Following 2 weeks in clean water, no residues could be detected in the fish (Rhodes 1980b).

3.2 Soil

In soil, hexazinone is dissipated by photodegradation and biodegradation to various metabolites and by leaching (U.S. Environmental Protection Agency 1982). Due to its relatively low vapor pressure, hexazinone does not volatilize to any appreciable extent (Ghassemi et al. 1981).

Radiolabeled hexazinone applied to soils in Delaware, Illinois, and Mississippi under field conditions at a rate equivalent to 3.3 lb/acre (3.7 kg/ha) had half lives of 1 month in Delaware, 2 months in Illinois, and 6 months in Mississippi. The half-lives of total radioactive residues (radiolabeled hexazinone and its metabolites) in the same soils were approximately 3 to 4 months in Delaware, 6 to 7 months in Illinois, and 10 to 12 months in Mississippi (Rhodes 1980a). Ghassemi et al. (1981 and 1982) reported DuPont studies evaluating the effect of hexazinone application rate on persistence in Keyport silt loam in Delaware. Hexazinone was applied at 4 rates from 1.8 to 20.0 lb a.i./acre (2.0 to 22.4 a.i. kg/ha) with samples collected initially and at 1, 6, and 8 months after treatment. Residue studies indicated that the half-life of hexazinone was about 1 month and was independent of application rate for the range of application rates and for the soil type studied. Ghassemi et al. (1981 and 1982) also reported persistence studies conducted with Velpar® Weed Killer (90W)* and Velpar® Gridball® by the USDA Forest Service Southern Forest Experiment Station. Velpar® Weed Killer (90W) was applied at rates of 1.8 and 3.6 lb/acre (2 and 4 kg/ha) to both clay and loamy sand in Alabama. The half-life of hexazinone was determined to be 4 to 6 weeks in the clay and less than 4 weeks in the loamy sand. The half-life of Velpar® Gridball® applied at rates of 1.8 and 3.6 lb/acre to a deep (100 inch) sandy soil was determined to be 14 weeks.

Hexazinone has been shown to photodegrade when applied to soil samples under laboratory conditions (Ghassemi et al. 1981 and 1982 and U.S. Environmental Protection Agency 1982). When radiolabeled hexazinone was incorporated into a thin-layer of soil at 10 ppm and placed under artificial sunlight for 6 weeks, the half-life of the parent hexazinone was estimated to be 37 days. By the end of the 6-week period, 40 percent of the parent ^{14}C-hexazinone remained in the soil. The rate of photodegradation in soil under natural field conditions would depend to a large extent on such environmental factors as light intensity and depth of incorporation of hexazinone in the soil.

Soil metabolism studies have demonstrated the biodegradation of hexazinone under aerobic conditions (Rhodes 1980a). Samples of Fallsington sandy loam and Flanagan silt loam were treated with radiolabeled hexazinone to achieve a concentration of 4 ppm hexazinone in the soil. In the soils kept under aerobic conditions, the half-life for hexazinone degradation was less than 4 months and the half-life of the total applied ^{14}C (hexazinone and metabolite) was reported to be greater than 6 months. No degradation or loss of

*90 percent active ingredient, soluble powder.

^{14}C activity was observed, however, when similarly treated soils were kept under anaerobic conditions for 60 days. Laboratory flask studies under aerobic conditions with these two soil types with ^{14}C-hexazinone at 4 ppm and 20 ppm demonstrated a rapid evolution of CO_2 following an initial lag period of 10 to 20 days. After 80 days of incubation, 45 to 75 percent of the total applied radioactivity had been given off as CO_2 in unsterilized soils compared to 0 to 10 percent in sterilized soils. These data, reported by Rhodes (1980a), are interpreted by the U.S. Environmental Protection Agency (1982), based on DuPont data, to be indicative of microbial, particularly fungal, activity.

Laboratory studies (Rhodes 1980a) indicate that hexazinone is mobile and thus readily leached in all of the soils studied. When the applied hexazinone was allowed to age for 30 days prior to percolation of water to measure leaching in a laboratory soil column, leaching rates were lower than for fresh hexazinone, indicating that the hexazinone metabolites may be less water soluble and/or more highly adsorbed to soil particles than the parent hexazinone (DuPont data in U.S. Environmental Protection Agency 1982). Studies conducted with Velpar® Gridball® by the U.S. Forest Service Southern Forestry Experiment Station (Ghassemi et al. 1981) on a deep sandy soil indicated that as the Gridball® pellets dissolved, the hexazinone material moved downward through the soil rather rapidly but moved laterally relatively slowly.

3.3 Water

Photodegradation, biodegradation, and dilution are the prime mechanisms for loss of hexazinone activity in aquatic systems. Studies with dilute aqueous solutions of hexazinone in the dark indicate that, at pHs of 5, 7, and 9, and at temperatures of 59, 77, and 98.6°F (15, 25 and 37°C), hexazinone does not degrade (<1% decomposition) for at least 5 to 8 weeks (Rhodes 1980b).

Hexazinone degrades readily in sunlight under a variety of aqueous conditions. Its degradation in natural waters is only slightly inhibited by presence of sediments.

Laboratory photodegradation studies were carried out by DuPont under both artificial and natural sunlight with ^{14}C-labeled hexazinone (Rhodes 1980b and U.S. Environmental Protection Agency 1982). Four aqueous solutions: (1) distilled water; (2) standard reference water comparable to "typical" surface water in the United States; (3) distilled water with anthraquinone photoinitiator, and; (4) distilled water with riboflavin photoinitiator, each of which contained 5 ppm hexazinone, were exposed to artificial light for 5 to 8 weeks. In addition, four aqueous solutions: (1) standard

reference water; (2) standard reference water containing riboflavin photoinitiator; (3) river water, and; (4) river water containing bottom sediments, each of which contained 5 ppm hexazinone, were exposed to natural sunlight for 5 weeks. In distilled water exposed to artificial light, hexazinone is slowly degraded with approximately 10 percent degradation after 5 weeks exposure. With both artificial and natural sunlight, decomposition is 3 times greater in standard reference water and in water with anthraquinone or with bottom sediments than it is in distilled water. Decomposition is 4 to 7 times greater in natural river and in distilled water with riboflavin than in distilled water alone.

The environmental fate of hexazinone in a small (approximately 5 hectares) watershed in Georgia was reported by Mayack et al. (1982). Residues of hexazinone and metabolites were monitored in a stream leading from the watershed after 10 percent a.i. hexazinone pellets were applied to portions (acreage unspecified) at a rate of 15 lb/acre (16.8 kg/ha). For the 8 months following application, most water samples had hexazinone levels below detection limits (1.0 ppb). Intermittent concentrations of 6 to 44 ppb were detected, primarily during the third and fourth month following hexazinone application.

4.0 Exposure And Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to hexazinone during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved hexazinone. Calculations of exposure to hexazinone in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to hexazinone are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Hexazinone Application

Available application data for hexazinone use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to hexazinone differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of hexazinone in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

The persistence of hexazinone in soil is 4 to 14 weeks and is independent of application rate. Hexazinone is readily dissipated from soil at a moderate rate by photodegradation, biodegradation, and leaching. The potential for incidental exposure of humans and wildlife to hexazinone is moderate following application.

4.2 Exposure

In 1982, liquid formulations of hexazinone were applied as a ground foliar spray at a maximum application rate of 4.0 lbs. a.i./acre. Gridball®, which is in pellet form, was also applied by aerial and by hand broadcast at a maximum rate of 2.0 lbs a.i./acre for both application methods.

Table 4-1

USDA Forest Service Application Data for Hexazinone (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Liquid (11)	Ground Foliar	7	77	6.1	6.5	6.1	8.1	Timber (10) Nursery (1)
Gridball (16)	Hand Broadcast	6.4	103	5.7	18.2	7	21.1	Timber (12) Wildlife (3) Range (1)
Gridball (2)	Aerial	8.5	17	4	1	4.5	1.5	Timber (2)

[a]Grand Total = 197

Source: Gross 1983

However, in toxicity studies described in Section 2, it is noted that hexazinone formulations such as Velpar® Gridball® pellets are not consumed orally and generally are not touched by animals. Since Gridball® is in pellet form, the herbicidal agent does not adhere to vegetation. Therefore, it is assumed that animals are not exposed to Gridball® pellets. In terms of observers for aerial application, it is assumed that no exposure, and consequently no dose, will occur since only pellets are applied by aerial means. For this analysis calculations in Table 4-2 that are based on ground spraying (e.g. backpack sprayer dose, and wildlife oral exposures) assume an application rate of 4.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of hexazinone and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of hexazinone to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational exposure estimates for hexazinone are based on exposures on a per pound per acre application rate multiplied by 4.0 lb/acre for backpack sprayers. The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent.

For ground applications at 4.0 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.16 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by hexazinone application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Hexazinone

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg		[b]
Observer	No dose calculated. See text.		
Backpack Sprayer	0.16 mg/kg		
Environmental Exposure			
Fish	Negligible residues. See text.		
Rabbits	0.806 mg/kg	Negligible	4.20 mg/kg
Deer	0.202 mg/kg	Negligible	5.76 mg/kg
Man	--[c]	--[c]	--[c]
(Fish)	NA[d]	NA	0.000 mg/kg
(Rabbits)	NA	NA	0.018 mg/kg (2 days)
(Deer)	NA	NA	0.023 mg/kg (2 days)
(Water)	NA	NA	0.000 mg/kg
(Berries)	NA	NA	0.000 mg/kg
(Mushrooms)	NA	NA	0.000 mg/kg
Toxicity Summary	Acute LD_{50} >2,000 mg/kg in rabbits	LC_{50} > 7.3 ppm in rats	Acute LD_{50}'s = 860 mg/kg (guinea pigs); 2,012 mg/kg (rats). NOEL = 200 ppm in rats and mice for chronic oral effects.
	Most studies, LC_{50} > 100 ppm for fish		

[a]Assumes ground foliar application at 4 lb active ingredient per acre. Assumes no aerially applied liquid.

[b]Occupational doses include all routes of exposure.

[c]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[d]NA represents nonapplicable data.

4.2.2 Environmental Exposures

Environmental exposures to man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the mixer/loaders or backpack sprayer. Animals in the target spray zone, however, are subject to dermal exposure. It was assumed that animals would not be sprayed directly by backpack applicators, and that only the animals' ventral surfaces would contact foliage that had been sprayed. Thus, approximately one half of the animals' body surface would contact sprayed vegetation, resulting in an exposure level equal to one half of that calculated for aerial application methods (aerial application amounts for exposure of the dorsal surface as well as ventral). Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 4.0 lb a.i./acre ground foliar spray:

Rabbits in Spray Zone: 0.806 mg/kg (1/2 x 0.403 mg/kg at 1 lb/acre application rate)

Deer in Spray Zone: 0.202 mg/kg (1/2 x 0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of hexazinone vapor would be neglible since the vapor pressure of hexazinone is reported to be low (Weed Science Society of America 1983). Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures to herbicides from immersion in streams

and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between spray zones and waterbodies, environmental exposures for aquatic organisms are expected to be zero. This is because it is assumed that backpack sprayers would not directly spray waterbodies and Gridball® would not be dropped into waterbodies. Although Gridball® leaches in soil, leaching tends to be downward, not lateral.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of hexazinone by workers on the spray unit during ground foliar applications would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume ground foliar application at 4.0 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 5.76 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 4.20 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of hexazinone in fish, deer, and rabbit.

Since negligible residues are assumed in water and since hexazinone is very rapidly photodegraded in water, fish tissue concentrations are assumed to negligible. Deer and rabbit tissue concentrations include hexazinone accumulated from both oral and dermal exposure to ground foliar treated areas. Tissue concentration estimates for deer and rabbit assume that dermally applied hexazinone is absorbed at a rate of 10 percent as discussed in Section 4.2.1 with regard to backpack sprayer absorption rates. It is also assumed that oral exposure of deer and rabbit to hexazinone lasts only for 2 days due to the relatively short environmental persistence of hexazinone and that 10 percent of the ingested material is assimilated into deer or rabbit muscle tissue. Deer tissue concentrations of hexazinone are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.202 mg/kg (dermal exposure) x 0.1 = 0.0202 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of 2-days feeding, or 5.76 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.152 mg/kg.

Total Deer Tissue concentration = 0.0202 mg/kg + 1.152 mg/kg = 1.1720 mg/kg (1.172 ppm).

Rabbit tissue concentrations of hexazinone are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.806 mg/kg (dermal exposure) x 0.1 = 0.0806 mg/kg.

Oral Exposure contribution assumes 10 percent absorption of 2-days feeding, or 4.20 mg/kg/day (oral exposure) x 0.1 x 2 days = 0.840 mg/kg.

Total Rabbit Tissue Concentration = 0.0806 mg/kg + 0.84 mg/kg = 0.9206 mg/kg (0.921 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to hexazinone from eating meat are derived:

Fish meat: negligible human oral exposure.

Deer meat: 1.172 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.023 mg/kg

Rabbit meat: 0.921 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.018 mg/kg

Water and plant consumption by man would result in negligible oral exposures to hexazinone since it is assumed that ground foliar spray zones would be posted and that water residues would be negligible.

4.3 Hazard Assessment

Toxicological properties of hexazinone to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rat inhalation results, guinea pig and rat acute oral LD_{50}'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for Forest Service application. Based on a NOEL of 200 ppm in a 2-year rat study, the U.S. Environmental Protection Agency (1982) has adopted a provisional acceptable daily intake (PADI) of 0.0125 mg/kg/day.

Small mammal studies have shown that hexazinone applied to the eye can be mildly to severely irritating and that hexazinone fed at rates up to 5,000 ppm in feed does not result in birth defects (in rats). Hexazinone is not mutagenic in most test systems and does not appear to be carcinogenic. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from hexazinone use, if proper care is taken during application.

Appendix A

Summary of Hexazinone Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Hexazinone	Honeybee (Insecta)	Surface absorption	48 hours	LC_{50} >60 µg/bee	Topical application to bees at 60 µg/bee (highest dose tested) resulted in only 10% mortality in a 48-hour test.	Schneider 1984 and DuPont undated
Hexazinone, Technical	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	LC_{50} = 151.6 ppm	95% percent confidence limits: 125.2 to 172.8 ppm.	DuPont undated and U.S. Environmental Protection Agency 1982
	Grass Shrimp (Palaemonetes pugio; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} between 56 and 100 ppm	Criterion for effect was death.	
	Fiddler Crab (Uca pugilator; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} >1,000 ppm	Criterion for effect was death.	
Hexazinone, Technical (95%)	Cladoceran (Daphnia sp.; Crustacea)	Surface absorption, static renewal bioassay	21-day life cycle study	LC_{50} = 33.1 ppm with 7-day renewal of hexazinone; 20 ppm < LC_{50} < 50 ppm with 3-day renewal of hexazinone	NOEL = 20 ppm NOEL = 10 ppm	Data reported in Mayack et al. 1982 and U.S. Environmental Protection Agency 1982
Hexazinone, Technical	Oyster Larvae (Crassostrea virginica; Mollusca)	Surface absorption static bioassay	48 hours	320 ppm< EC_{50} < 560 ppm	Criterion for effect was a reduction in number of normal embryos.	DuPont undated and U.S. Environmental Protection Agency 1982
Hexazinone (pellets, 10% a.i.)	Aquatic invertebrates	Surface absorption field exposure	Intermittent exposure to levels of 6 to 44 ppb in water	No major alterations in aquatic macroinvertebrate species composition or diversity were detected.	Sampling was conducted for 8 months following application of pellets in late April to a forested watershed at a rate of 15 lb/acre (16.8 kg/ha).	Mayack et al. 1982

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Hexazinone (pellets, 10% a.i.)	Terrestrial invertebrates	Surface absorption field exposure	--[a]	No major community change in soil microarthropods.	Sampled approximately 3 1/2 months after application of pellets in late April to a forested watershed at a rate of 15 lb/acre (16.8 kg/ha).	Mayack et al. 1982
Hexazinone	Soil fungi (including the genera Penicillium, Aspergillus, Trichoderma, Mucor, Fusarium) and soil bacteria	Incorporated into soil cultures in laboratory	8 weeks	10 ppm hexazinone did not reduce soil population counts of fungi or bacteria. At 100 ppm, hexazinone showed little to no fungitoxicity.	--	Rhodes et al. 1980
Hexazinone	Soil bacteria	Incorporated into soil cultures in laboratory	5 weeks	Five and 20 ppm hexazinone did not adversely affect soil nitrification processes.	--	Rhodes et al. 1980
Hexazinone	Common algae (plants) Cladophora clomerata, Rhizochonium hieroglyphium,	--	--	Inhibition at 0.5 ppm	--	Fowler 1977 in Mayack et al. 1982
	Vaucheria dichotoma	--	--	Inhibition at 1.0 ppm	--	

[a]Data not available or not applicable.

Appendix B

Summary of Hexazinone Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Hexazinone	Rainbow trout	Surface absorption, static bioassay	96 hours	320 ppm < LC_{50} <420 ppm	NOEL = 240 ppm. Temperature 52.8°F (11° ± 1.0°C). Average weight 0.035 oz (1 gram).	DuPont undated and U.S. Environmental Protection Agency 1982
	Bluegill	Surface absorption, static bioassay	96 hours	370 ppm < LC_{50} <420 ppm	NOEL = 370 ppm	DuPont undated and U.S. Environmental Protection Agency 1982
	Fathead Minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 274 ppm	95% confidence limits = 207 to 361 ppm; NOEL = 160 ppm.	
Hexazinone	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 322 ppm	--[a]	Schneider and Kaplan 1983
Hexazinone	Gold orfe	Surface absorption, static bioassay	96 hours	LC_{50} = 340 ppm	--	
Hexazinone (Velpar®L) (25% liquid)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 925 ppm	95% confidence limits of 782 to 1,049 ppm	Schneider and Kaplan 1983
Hexazinone (Velpar®)	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	0.7 grams, 54°F (12°C)	Johnson and Finley 1980
	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} > 100 ppm	0.8 grams, 72°F (22°C)	
Hexazinone (^{14}C)	Bluegill	Surface absorption	4 weeks	No effect on fish exposed to 0.01 and 1.0 ppm.	--	Rhodes 1980b
Hexazinone, Technical	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 505 ppm	95% confidence limits of 450 to 538 ppm. Fish 1.26 inches (3.2 cm) long kept at 68°F (20° ± 0.2°C), dissolved oxygen of 5.7 to 9.5 ppm and pH 7.1 to 7.3.	Data cited in U.S. Environmental Protection Agency 1982

[a]Data not available or not applicable.

Appendix C

Summary of Hexazinone Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Hexazinone, Technical	Bobwhite Quail	Oral, acute, single dose	(Acute)	LD_{50} = 2,258 mg/kg	95% confidence levels = 1,628 to 3,130 mg/kg. By oral intubation to fasted 20-week old males and females at 0, 398, 631, 1,000, 1,590, and 2,510 mg/kg. Birds observed for 2-weeks for mortality and toxic effects.	U.S. Environmental Protection Agency 1982; DuPont undated; and DuPont data cited in Ghassemi et al. 1981 and 1982
Hexazinone	Bobwhite Quail	Oral, subchronic	5 days (of an 8-day study)	LC_{50} >10,000 ppm	Fed in diet to 14-day old quail at 0, 625, 1,250, 2,500, 5,000, 10,000 ppm for first 5 days of an 8-day study. Birds observed daily for mortality and toxic effects.	DuPont undated and PuPont data cited in Ghassemi et al. 1981 and 1982
Hexazinone, Technical	Bobwhite Quail	Oral, subchronic	5 days (of an 8-day study)	LC_{50} > 5,000 ppm	Fed in diet to 14-day old birds at 0, 156, 312, 625, 1,250, 2,500 and 5,000 ppm in diet.	Data cited in U.S. Environmental Protection Agency 1982
Hexazinone	Mallard (Ducklings)	Oral, subchronic	5 days (of an 8-day study)	LC_{50} >10,000 ppm	Fed in diet to 1-month old ducks at 0, 625, 1,250, 2,500, 5,000, and 10,000 ppm for first 5 days of an 8-day study. Birds observed daily for mortality and toxic effects.	DuPont undated and DuPont data cited in Ghassemi et al. 1981 and 1982

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Velpar® Gridballs®	Black-capped chickadee Ring-necked pheasant	Gridballs® placed near feeding site in captivity	3 weeks	Birds did not respond to Gridball® pellets.	--[a]	Richmond 1979
Velpar® Gridballs®	Black-capped chickadee, Ring-necked pheasant, White-breasted nuthatch, Downy woodpecker, Cardinal, Song sparrow, Rose-breasted grosbeak	Gridballs® placed near feeding sites in a natural environment.	1 to 3 weeks	Birds did not respond to Gridball® pellets.	--	Richmond 1979

[a]Data not available or not applicable.

Appendix D

Summary of Hexazinone Toxicity in Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Hexazinone (0.5% liquid)	Rat 205-228 gm Sprague-Dawley (5 males and 5 females)	Dosed with 5,000 mg/kg; route not specified.	$LD_{50} > 5,000$ mg/kg	--[a]	U.S. Environmental Protection Agency 1982
Hexazinone (66% wettable powder suspension in corn oil)	Rat (male ChR:CD)	Single dose by intragastric intubation on the basis of 3,200, 3,500, 4,000, 4,500, 5,000, 6,000, and 7,500 mg/kg body weight.	LD_{50} = 4,495 mg/kg	95% confidence limits of 3,808 and 5,263 mg/kg	U.S. Environmental Protection Agency 1982
Hexazinone (unformulated)	Guinea pig (male, albino)	Single dose of a suspension in 15:85 acetone: corn oil by intubation to 10 animals per dose. Animals observed 14 days.	LD_{50} = 860 mg/kg body weight with 95% confidence limits of 450 and 1,260 mg/kg body weight. Lethargy, ataxia, convulsions, weight loss, protration, salivation, lacrimation, and ruffled fur observed.	--	Newton and Dost 1981; Weed Science Society of America 1983; and Ghassemi et al. 1981
Hexazinone (unformulated)	Rat (male ChR:CD)	Single dose of a suspension in 15:85 acetone: corn oil by intubation to 10 animals per dose. Animals observed for 14 days.	LD_{50} 1,690 mg/kg with 95% confidence limits of 1,560 and 1,880 mg/kg body weight; observed lethargy, ataxia, convulsions, prostration, salivation, and ruffled fur.	--	Schneider and Kaplan 1983
Hexazinone (10% pellet formulation; suspended in corn oil)	Rat (male)	Single oral dose as suspension in corn oil to 10 animals; observed for 14 days.	$LD_{50} > 7,500$ mg/kg. Slight weight loss, no mortalities, no pathologic changes.	--	U.S. Environmental Protection Agency 1982 and Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
Hexazinone (unformulated)	Dog (beagle)	Single dose in gelatin capsules administered one dog each at levels of two 2,250 mg/kg body weight and 3,400 mg/kg body weight. Single dose of 15% aqueous suspension administered one dog at level of 1,000 mg/kg body weight. Observed for 14 days after dosing.	ALD > 3,400 mg/kg body weight; emesis, tremors salivation, and rapid respiration observed within minutes to hours of dosing. Lacrimation and polyurea on day of dosing. Diarrhea 1 day after dosing.	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Rat (male, Crl:CD)[b]	Single dose of corn oil suspension administered one animal per dose. Dose not specified. Animals weighed and observed for 14 days.	ALD = 1,500 mg/kg body weight; prostration, irregular respiration, and half-closed eyes observed on day of dosing; in addition, apprehension, incoordination, and convulsions observed at lethal doses.	--	Schneider and Kaplan 1983
Hexazinone (89.3% a.i.)	Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to one animal per dose level. Dose not specified. Animals observed for 14 days.	ALD= 5,000 mg/kg body weight; congestion, stained nose and mouth, and transient weight loss observed at sublethal doses; rapid and labored respiration, pallor, prostration, lethargy, half-closed eyes, and convulsions at lethal dose.	--	Schneider and Kaplan 1983

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
Hexazinone (unformulated)	Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 10 animals per dose level. Dose not specified. Animals observed for 14 days.	LD_{50} = 2,012 mg/kg body weight. Observed tremors and convulsions on day of dosing; observed weakness and wet perinea on day after dosing; observed weight loss for 1 to 2 days after dosing.	--	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rat (male, Crl:CD)	Single dose administered by intubation to 10 animals per dose. Dose not specified. Observed for 14 days. Three animals from highest doses and two animals from other doses examined for changes in gross pathology.	LD_{50} = 7,080 mg/kg body weight with 95% confidence limits of 6,666 and 8,753 mg/kg body weight. Observed nondose-related prostration, wet and stained perinea and nasal areas, lethargy, congestion, irregular respiration, and convulsions. Gross examination revealed no compound-related pathologic changes.	--	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rat (male, Crl:CD)	Single dose of an aqueous solution administered by intubation to 10 animals per dose. Dose levels were 6,000 to 9,000 ppm. Survivors were weighed and observed for 14 days, then sacrificed for examination of gross pathology.	LD_{50} = 6,887 mg/kg body weight. Observed slight to moderate weight losses, weakness, half-closed eyes, wet and stained perinea, stained face, labored breathing, ataxia, and convulsions. At gross examination observed discolored and heavy lungs at all doses and slightly heavy liver in one rat at 9,000 ppm.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Continued)		
Hexazinone (66% wettable powder)	Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 10 animals per dose. Dose levels include 3,200, 5,000, and 6,000 ppm. Observed for 14 days. Two animals from each dose examined for changes in gross pathology.	LD_{50} = 4,495 mg/kg body weight with confidence limits of 3,808 and 5,263 mg/kg body weight; observed prostration, salivation, piloerection, wet and stained perinea, stained mouth and nose, half-closed eyes, labored breathing, tremors, and convulsions. Observed no treatment-related changes in gross pathology.	--	Schneider and Kaplan 1983
Hexazinone (10% Gridball®)	Rat (male Crl:CD)	Single dose of corn oil suspension of 10% Gridball® administered to one group of 10 animals. Observed 14 days. Two animals examined for changes in gross pathology.	LD_{50} > 7,500 mg/kg body weight. Observed slight weight loss and no mortalities; observed no treatment-related changes in gross pathology.	--	Schneider and Kaplan 1983
Hexazinone (20% Gridball®)	Rat (male and female, Crl:CD)	Single dose of corn oil suspension administered to groups of 5 fasted rats per sex. Dose not specified. Animals weighed and observed for 14 days, then sacrificed and examined for changes in gross pathology.	LD_{50} > 5,000 mg/kg body weight. Observed in females but not in males, prostration, stained and wet perinea, stained face, weakness and slight weight loss. Observed lung and liver abnormalities in both sexes. Observed discolored thymus in one female.	--	Schneider and Kaplan 1983
Hexazinone (20% Gridball®)	Rat (male and female, Crl:CD)	Single dose of corn oil suspension administered to 10 animals per sex per dose. Dose not specified. All animals weighed and observed for 14 days, then sacrificed and examined for changes in gross pathology.	Males: LD_{50} = 11,798 mg/kg body weight with confidence limits of 10,721 and 13,155 mg/kg. Females: LD_{50} = 6,352 mg/kg body weight with confidence limits of 5,243 and 10,371 mg/kg. Observed prostration, half-closed eyes, stained perinea, weakness salivation, weight loss, convulsions at lethal doses, and gross pathological changes in lungs, liver, stomach, thymus, and occasionally in salivary lymph nodes, spleen and brain.	--	Schneider and Kaplan 1983

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity (Concluded)		
Hexazinone (unformulated)	Rat (male, Crl:CD)	Administered 300 mg/kg/day by intubation as a 5% corn oil suspension 5 times a week for 2 weeks to 6 rats per group. Three tests and 3 control rats sacrificed at 4 hours and 14 days after the last dose and examined for changes in microscopic pathology.	All rats survived. No clinical evidence of toxicity. No apparent treatment-related changes in microscopic pathology.	--	Schneider and Kaplan 1983
Hexazinone (89.3% a.i.)	Rat (male, Crl:CD)	Administered 300 mg/kg/day by intubation as a 5% corn oil suspension 5 times a week for 2 weeks to 6 rats per group. Three tests and 3 control rats sacrificed at 4 hours and 14 days after the last dose and examined for changes in microscopic pathology.	All rats survived. Observed slight weight loss. No apparent treatment-related changes in microscopic pathology.	--	Schneider and Kaplan 1983
Hexazinone (89.3% a.i.)	Rat (male, Crl:CD)	Administered 1,000 mg/kg/day by intubation as a 6% corn oil suspension 5 times a week for 2 weeks to 10 rats per group. Five tests and 5 control rats sacrificed at 4 hours and 14 days after the last dose and examined for changes in microscopic pathology.	All rats survived. Observed slight weight loss, wet and stained perinea, congestion, salivation, and chromodacryorrhea and were treatment related. Increased liver weight and slightly to moderately enlarged hepatocytes were noted at the end of the treatment period and were treatment-related.	--	Schneider and Kaplan 1983
			Acute Intraperitoneal Toxicity		
Hexazinone (unformulated)	Rat (male, Crl-CD)	Single dose of test material suspended in saline injected intraperitoneally into 10 animals per dose levels. Animals weighed and observed for 14 days.	LD_{50} = 530 mg/kg body weight with 95% confidence range of 380-570 mg/kg body weight. Observed initial weight loss, pallor, salivation, bleeding around nose and mouth, labored irregular respiration, lethargy, prostration, ruffled fur, and colonic convulsions.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity		
Hexazinone (90% soluble powder)	Rabbit (male)	Single dose of 60 ml of 24% aqueous suspension applied to intact skin on shaved trunk area of 3 rabbits, covered with gauze and occlusive wrappings for 24 hours followed by a post-treatment period.	ALD >5,278 mg/kg body weight. Observed mild erythema in one rabbit; by 24 hours after treatment, all animals had normal appearance and behavior.	--	Ghassemi et al. 1981 and Schneider and Kaplan 1983
Hexazinone (90% soluble powder)	Rabbit (male)	Groups of 6 rabbits treated with 0, 68, and 680 mg a.i. per kg body weight applied to intact skin. Covered with gauze and occlusive wrappings for 6 hours per day for 10 days. Clinical and pathological examination conducted on all animals at sacrifice. Subsequently, groups of 6 rabbits were treated in the same way with 0, 35, 150, and 770 mg/kg a.i.per kg body weight; blood was analyzed on days 14, 28, and 53 after treatment.	Transiently elevated glutamic-pyruvic transaminase activity at the 680 and 770 mg a.i. per kg body weight doses; no evidence of liver or other tissue injury.	--	Ghassemi et al. 1981 and Schneider and Kaplan 1983
Hexazinone (60% dry flowable)	Rabbit (male albino)	0.5 gm applied to intact and abraded skin under gauze	Not a primary skin irritant with score of 0.75.	--	U.S. Environmental Protection Agency 1982
Hexazinone (0.5% liquid)	Rabbit (5 males and 5 females, New Zealand White)	2 g/kg dose applied to abraded skin and placed under occlusive wraps for 24 hours. Observations at 1, 3, 7, 10, and 14 days.	LD_{50} >2 g/kg	--	U.S. Environmental Protection Agency 1982
Hexazinone (0.5% liquid)	Not specified	Applied 0.5 ml to 2 abraded and 2 intact skin sites per animal for 24 hours under occlusive wrap. Observations at 24 and 72 hours.	No erythema or edema at any site at 72 hours.	--	U.S. Environmental Protection Agency 1982

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Continued)		
Hexazinone (unformulated)	Guinea pig (male)	Applied 0.05 ml of 25 and 50% suspensions in distilled water to shaved intact shoulder skin of 10 guinea pigs followed by examination after 24 and 48 hours. Induction for sensitization was by 4 weekly intradermal injections of 0.1 ml of 1% test material in dimethyl phthalate. Thirteen days after the last injection the animals were topically challenged with 0.05 ml of 25 and 50% aqueous suspensions then examined at 24 and 48 hours.	No skin irritation or sensitization.	--	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rabbit (male)	Treated one rabbit each with a single dose of 2,250, 3,400, 5,000, or 7,500 mg/kg body weight and subsequently 5 rabbits with one dose of 7,500 mg/kg body weight. The intact skin was treated, covered by gauze and occlusive wrappings for 24 hours and observed for 14 days post-treatment.	$LD_{50} > 7,500$ mg/kg body weight. No mortalities or adverse clinical effects.	--	Schneider and Kaplan 1983
Hexazinone (66% dry flowable)	Rabbit, (male and female, New Zealand White)	Five rabbits per sex treated with a single dose of 2,000 mg/kg body weight to abraded skin. Covered with gauze and occlusive wrappings for 24 hours. Observed 14 days then sacrificed. Two animals per sex submitted to gross and microscopic examination of treated skin.	$LD_{50} > 2,000$ mg/kg body weight. Moderate skin irritation at 24 hours, normal by 14 days. No gross or microscopic pathological abnormalities.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Continued)		
Hexazinone (66% wettable powder)	Rabbit (male)	Treated 6 rabbits with a 63% aqueous paste at a dose of 9,100 mg/kg body weight. Applied to intact skin, covered with gauze and occlusive wrappings 24 hours, then observed for 14 days.	LD_{50}> 9,100 mg/kg body weight. (<6,000 mg/kg a.i.); moderate irritation at 24 hours; normal at 7 days.	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Rabbit (male, New Zealand White)	Treated 2 intact and 2 abraded areas of skin on each of 6 rabbits with 0.5 gm of solid test material moistened with saline. Treatment sites covered 24 hours, then washed and scored according to Draize after 48, 72 and 96 hours.	Not a primary skin irritant. Draize scores from 0.5 to 1.5.	Draize score >5.0 required for primary irritant classification.	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rabbit (male, New Zealand White)	Treated 2 intact and 2 abraded areas of skin on each of 6 rabbits with 0.5 g moistened with saline then covered with gauze for 24 hours. Treatment sites scored according to Draize after 24 and 72 hours.	Not a primary skin irritant; Draize scores from 0.0 to 0.75.	--	Schneider and Kaplan 1983
Hexazinone (66% wettable powder)	Rabbit (male)	Treated 2 intact and 2 abraded areas of skin on each of 6 rabbits with 0.5 g moistened with saline then covered with gauze for 24 hours. Treatment sites scored according to Draize after 24 and 72 hours.	Not a primary skin irritant. Draize scores = 0.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Dermal Toxicity (Concluded)		
Hexazinone (25% liquid)	Guinea pig (male)	Applied 0.05 ml of 100% test material and 10% solution of test material in distilled water to shaved intact shoulder skin then examined after 24 and 48 hours. Sensitization induced by 4 weekly intradermal injections of 0.1 ml of 1% test material in saline in 10 animals. Thirteen days after the last injection the animals were topically challenged with 0.05 ml of 100% test material and 10% test material in distilled water then examined at 24 and 48 hours.	Mild transient irritation at 100%; no sensitization.	--	Schneider and Kaplan 1983
			Eye Irritation		
Hexazinone (unformulated)	Rabbit	Applied to right eyes of 2 rabbits; 10 mg of powder; 1 eye washed with water and 1 eye not washed. Eyes examined after 1 and 4 hours and 1, 2, 3, 7, and 9 days.	Eye irritant. Caused moderate corneal injury and moderate conjunctivitis with no iritic effect in unwashed and slightly less in washed eyes. Corneal damage reverted in 9 days in unwashed eye and in 7 days in washed eye.	--	Newton and Dost 1981; Ghassemi et al. 1981; and Schneider and Kaplan 1983
Hexazinone (90% soluble powder)	Rabbit	Right eyes of 6 rabbits treated with 0.1 ml powder; eyes examined 24, 48, and 72 hours after treatment.	Eye irritant. Corneal and conjunctival damage in 6 of 6 rabbits through 72 hours.	--	Ghassemi et al. 1981 and Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Eye Irritation (Continued)		
Hexazinone (90% soluble powder)	Rabbit	Repeated exposure. Right eyes of 5 rabbits treated with 1 mg powder on first day of treatment. Each of the next 4 days, 1 rabbit removed from treatment while the remaining treated as on day 1. Eyes not washed, but examined at 4 hours and at 1, 2, 3, 4, 5, 7, and 14 days.	Three of 5 eyes had localized corneal opacity, minimal conjunctivitis, and no iritic effect. All effects reversible within 3 to 14 days. No cumulative effects.	--	Ghassemi et al. 1981 and Schneider and Kaplan 1983
Hexazinone (66% wettable powder)	Rabbit	Treated right eyes of 6 rabbits with 0.1 ml (21.3 mg); eyes examined 1, 2, 3, 7, and 14 days after treatment.	Eye irritant. Three of 6 rabbits with corneal opacity on day 7 and 2 to 6 rabbits on day 14.	--	U.S. Environmental Protection Agency 1982
Hexazinone (0.5% liquid)	Rabbit (New Zealand White)	Left eye of 9 rabbits treated with 0.1 ml; 6 unwashed and 3 washed; observations at 24, 48, and 72 hours and days 4 and 7.	No corneal opacity, iritis or conjunctive irritation at any observation period.	--	U.S. Environmental Protection Agency 1982
Hexazinone (unformulated)	Rabbit (New Zealand White)	Treated right eyes of 9 rabbits with 0.1 ml (42 mg). Three eyes washed and 6 unwashed. Examined at 1, 2, 3, 4, 7, 14, 21, and 28 days and scored according to Draize.	Observed in 6 of 6 unwashed eyes mild to moderate corneal cloudiness and severe conjunctivitis. Observed in 5 of 6 unwashed eyes moderate iritis that reversed to normal in 2 of 6 eyes within 14 days. In 4 of 6 unwashed eyes corneal injury persisted through 28 days. In washed eyes, observed slight to mild corneal cloudiness, moderate iritis, and mild to severe conjunctivitis. All washed eyes normal in 21 days.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Eye Irritation (Continued)		
Hexazinone (89.3% a.i.)	Rabbit	Applied 10 mg to right conjunctival sac of 2 rabbits. One washed eye and 1 unwashed eye examined after 1 and 4 hours and 1, 2, 7, and 14 days.	Mild corneal opacity persisted 14 days in both eyes. Observed transient mild to moderate iritis and moderate conjunctivitis in both eyes.	--	Schneider and Kaplan 1983
Hexazinone (90% wettable powder diluted in water to 4% a.i.)	Rabbit	Applied 0.1 ml of test solution to right conjunctival sac of 2 rabbits. One washed and 1 unwashed eye examined after 1 and 4 hours and 1, 2, and 3 days.	Moderate to mild conjunctival irritation in unwashed eye normal in 3 days. Minimal conjunctivitis in washed eye normal in 1 day.	--	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rabbit	Treated right eyes of 6 rabbits with 0.1 ml of test material and examined eyes at 24, 48, and 72 hours.	Formulation is an eye irritant. Corneal, iritic and conjunctival injury in 6 of 6 rabbits through 72 hours.	--	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rabbit	Applied 0.1 ml of test material to right conjunctival sac of 2 rabbits. Examined 1 washed and 1 unwashed eye after 1 and 4 hours and 1, 2, 3, 7, and 14 days.	Moderate corneal damage; moderate iritis with flare, and severe to moderate conjunctival irritation in unwashed eyes with slightly less severe effects in washed eyes. All effects reverted to normal in 14 days in unwashed eyes and in 7 days in washed eyes.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Eye Irritation (Continued)		
Hexazinone (25% liquid)	Rabbit	Applied 0.1 ml of test material to right eyes of 9 rabbits. Three washed eyes and 6 unwashed eyes examined at 1, 2, 3, 4, 7, 10, 14, 17, and 21 days and scored according to Draize.	Mild to severe corneal irritation, slight to moderate iritis, and severe conjunctivitis in unwashed eyes with corneal effects and conjunctivitis lingering at 21 days. Mild to moderate corneal irritation, moderate to severe conjunctivitis and slight iritis in washed eyes. All cleared except slight corneal opacity in 1 eye at 21 days.	--	Schneider and Kaplan 1983
Hexazinone (25% liquid)	Rabbit	Applied 0.1 ml of test material to right conjunctival sacs of 3 rabbits and not washed and of 3 rabbits followed by a tap water wash. Also applied 0.1 ml of test material diluted to 4% a.i. in eyes of 3 rabbits and not washed. All eyes examined after 1 and 6 hours and 1, 2, 3, 4, 7, 14, 21, 29, and 35 days by opthalmoscope and biomicroscope (U.K. Procedure).	In 100% test material/unwashed eyes observed severe to moderate corneal cloudiness with moderate iritis and conjunctivitis. Corneal effects persisted 35 days. Iritic and conjunctival effects reverted in 21 days. In 100% test material/washed eyes, observed mild to moderate corneal moderate cloudiness and iritis and conjunctivitis. Corneal effects persisted 35 days in 1 rabbit. All effects reverted in 21 days in 2 rabbits.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Eye Irritation (Concluded)		
Hexazinone (25% liquid diluted in water to 4% a.i.)	Rabbit	Applied 0.1 ml to right conjunctival sac of 2 rabbits. One washed and 1 unwashed eye examined after 1 and 4 hours and 1, 2, and 3 days.	Mild to minimal conjunctival irritation and no corneal or iritic involvement in unwashed and washed eyes. Unwashed eye normal in 3 days and washed eye in 1 day.	--	Schneider and Kaplan 1983
Hexazinone (5% liquid)	Rabbit	Applied 0.1 ml to right eyes of 9 rabbits; 3 washed and 6 unwashed; examined after 1, 2, 3, 4, 11, and 14 days and scored according to Draize.	Mild corneal cloudiness, moderate iritis and moderate conjunctivitis observed in all unwashed eyes. All effects reversed within 14 days. Slight corneal cloudiness in 1 washed eye and moderate conjunctivitis in 3 washed eyes. All effects reverted in 4 days.	--	Schneider and Kaplan 1983
Hexazinone (1.25% liquid)	Rabbit (New Zealand White)	Applied 0.1 ml to lef eyes of 9 rabbits; 3 washed and 6 unwashed eyes examined at 24, 48, and 72 hours and scored according to Draize.	No ocular irritation.	--	Schneider and Kaplan 1983
			Inhalation Toxicity		
Hexazinone (unformulated)	Rat (male, ChR:CD)	Single one hour exposures of groups of 10 rats in a chamber to atmosphere of test material. Animals weighed and observed for 14 days. Concentrations were 0, 2.94, 5.14, and 7.48 mg/l with mass median diameter of 3.5 μ and at least 46.3% of particles less than 3.2 μm.	ALD > 7.48 mg/l; 100% survival; irregular respiration and salivation. No deaths.	--	Ghassemi et al. 1981 and Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Inhalation Toxicity (Concluded)		
Hexazinone (90% wettable powder)	Rat (male, ChR:CD)	Head only exposure in a chamber with an average concentration of 2.0 mg/l dust, 6 hr/day for 15 days with 2 days rest after 5th and 10th exposure. Mass median diameter was 7.85 μm with 28.7% of particles less than 3.2 μm.	Rats survived with no significant clinical or histopathological findings.	--	Ghassemi et al. 1981 and Schneider and Kaplan 1983
Hexazinone (0.5% liquid)	Rat (5 male and 5 female Sprague-Dawley, 261 to 338g)	Four hour exposure to 5.0 to 5.5 ppm. Normal concentration in a 100-liter glass and stainless steel chamber; airflow 16.7 1/min.; temperature 77 to 79°F; relative humidity 49 to 60%.	Normal behavior. Toxicity Category IV.	--	U.S. Environmental Protection Agency 1982
Hexazinone (90% soluble powder)	Rat (male, Crl:CD)	Single 1 hour head only exposure of 6 rats per group to atmospheres of test material Concentrations were 0, 4.1, 6.1 and 7.3 mg/l with mass median diameters of 10.5 and 6.0 μm in the mid and high concentrations and 28.3 and 18.7% of particles less than 3.2 μm in the mid and high concentrations, respectively. Rats weighed and observed for 14 days.	ALD > 7.3 ppm; no deaths.	--	Schneider and Kaplan 1983
Hexazinone (90% wettable powder)	Rat (male, Crl:CD)	Repetitive head only exposure of 10 rats per group for 6 hours a day, 5 days a week for 3 weeks. Concentrations were 0 or 2.0 mg/l. Mass median diameter of particles was 7.85 μm with 28.7% < 3.2 μm. Animals observed for 14 days post-exposure. At sacrifice 5 test and 5 control rats examined for changes in gross and microscopic pathology.	No deaths; no treatment related clinical or pathological abnormalities.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity		
Hexazinone (unformulated)	Rat (male, ChR:CD)	300 mg/kg/day by intubation; 5 days per week for 2 weeks	No deaths. No evidence of cumulative toxicity.	No clinical or histopathologic changes.	Weed Science Society of America 1983; Newton and Dost 1981 1981; and Ghassemi et al. 1981
Hexazinone (unformulated)	Rat (male, Crl:CD)	1,000 mg/kg/day by intubation 5 days per week for 2 weeks to groups of 10 rats.	No deaths. Compound-related increased liver weight and larger hepatocytes. Wet and stained perinea, congestion, slight weight loss, and salivation.	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Rat (weanling males and females; Crl:CD)	Fed 16 rats per sex per group 0, 200, 1,000, and 5,000 ppm in the diet for 90 days. Clinical pathological observations conducted at 30, 60, and 90 days.	NOEL = 1,000 ppm; decreased body weight and food efficiency at 5,000 ppm in both sexes. No other behavioral, nutritional, clinical, biochemical, or pathological evidence of toxicity observed.	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Dog (male and female beagle, young adults)	Fed 4 dogs per sex per group 0, 200, 1,000, and 5,000 ppm in the diet for 90 days. Clinical pathological examinations were conducted at 30, 60, and 90 days.	NOEL = 1,000 ppm; at 5,000 ppm reduced body weight gain, decreased food consumption elevated alkaline phosphatase, lower albumin: globulin ratios, and increased liver weight. No histopathologic, behavioral, clinical, or other nutritional, biochemical or gross pathological evidence of toxicity observed.	--	U.S. Environmental Protection Agency 1982; Newton and Dost 1981; Ghassemi et al. 1981; and Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Concluded)		
Hexazinone (unformulated)	Mice (male and female, Crl:CD-1)	Fed 10 mice per sex per group 0, 250, 500, 1,250, 2,500, 5,000, and 10,000 ppm in diet to weanlings for 8 weeks.	NOEL = 5,000 ppm; increased liver weight in both sexes 10,000 ppm; no other clinical, behavioral, nutritional, or pathological signs of toxicity.	--	Ghassemi et al. 1981 and Newton and Dost 1981
Hexazinone (unformulated)	Hamster (male and female engle)	Fed 0 to 10,000 ppm in diet to weanlings for 8 weeks.	No mortality or clinical signs of toxicity.	--	Ghassemi et al. 1981 and Newton and Dost 1981
Hexazinone (Gridball® pellets)	Cottontail rabbits, prairie voles, white-tailed deer, Norway rats and greys squirrels	Two to 100 Gridball® pellets for 3 to 5 weeks placed in feeding territories in natural habitat.	No mortality or unusual behavior.	--	Richmond 1979
Hexazinone (Gridball® pellets)	Racoons, skunks, opossum, and prairie voles	Two to 20 Gridball® pellets for 3 to 5 weeks placed in feeding area of live-trapped animals.	No mortality or unusual behavior; nibbling and and urine marking by prairie voles caused no adverse effects.	--	Richmond 1979

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity		
Hexazinone (90% wettable powder)	Rat (male and female, weanling, ChR:CD)	Fed 0, 200, 1,000, and 2,500 ppm in diet for 2 years to 36 rats per sex per group. Clinical pathology evaluated at 1, 2, 3, 6, 9, 12, 18, and 24 months. Interim sacrifice at 12 months.	NOEL = 200 ppm; body weight gain and food efficiency decreased in both sexes at 2,500 ppm and in females at 1,000 ppm; food consumption decreased in males at 2,500 ppm; leukocytosis and eosinophilia in males at 2,500 ppm. Increased urine alkalinity in both sexes at 2,500 ppm. No behavioral, clinical, pathological or other nutritional and biochemical evidence of toxicity observed.	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Hamsters (male and female)	5,000 ppm for 48 weeks in the diet.	No adverse effects reported due to test material administration.	Infections disease interrupted the study at 48 weeks.	Newton and Dost 1981
Hexazinone (unformulated)	Mice (male female, weanling, Crl:CD-1)	Fed 80 mice per sex per group 0, 200, 2,500, and 10,000 ppm in diet for 24 months. Hematology conducted at 1, 3, 6, 12, 18, and 24 months.	NOEL = 200 ppm; no behavioral, clinical, or nutritional signs of toxicity observed. Nonneoplastic hepatocellular effects observed in both sexes at 10,000 ppm and in males at 2,500 ppm.	--	Schneider and Kaplan 1983

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Teratology, Reproduction and Fertility (Concluded)		
Hexazinone (unformulated)	Rat (male and female, Crl:CD)	Fed 6 rats per sex per group 0, 200, 1,000 and 5,000 ppm through maturation, production and weaning of 1 litter.	No adverse effect on reproduction or lactation. Weanlings in the 5,000 ppm group had lower body weight than control.	--	Schneider and Kaplan 1983
Hexazinone (90% wettable powder)	Rat (male and female, ChR:CD)	Fed 0, 200, 1,000, and 2,500 ppm in diet for three generation, 3 litter study. First groups of 20 rats per sex were fed through maturation, reproduction and weaning of an F_1 litter. Groups of 20 F_1 rats per sex were fed as above through an F_2 litter. Groups of 20 F_2 weanlings per sex were fed as above through an F_3 litter.	NOEL = 1,000 ppm. No adverse effect on reproduction or lactation. The average body weights of the 2,500 ppm group F_2 and F_3 pups were lower than the control group at weaning.	--	Newton and Dost 1981; Ghassemi et al. 1981; and Schneider and Kaplan 1983
Hexazinone (unformulated)	Rat (female ChR:CD)	Fed 0, 200, 1,000, and 5,000 ppm in diet from day 6 through day 15 of gestation. Females sacrificed on 21st day of gestation and examined with fetuses.	No evidnce of embryo-toxicity, teratogenicity or clinical signs of toxicity except slightly decreased maternal food consumption and body weight gain at 5,000 ppm. Maternal weight slightly depressed at 1,000 ppm.	--	U.S. Environmental Protection Agency 1982 and 1983; Ghassemi et al. 1981; and Schneider and Kaplan 1983
Hexazinone (unformulated)	Rabbit (female)	Artifically inseminated then dosed by intubation a 0.5% methocel suspension of test material at concentrations of 0, 20, 50, or 125 mg/kg/day to 17 pregnant rabbits per group on days 6 through 19 of gestation. Pups delivered by cesarean on day 29.	NOEL = 125 mg/kg for embryo-fetal toxicity and teratogenicity; no gross pathology in test dams; no change in pregnancy rate uterine weight, corpora implantations, fetal viability, and size.	--	U.S. Environmental Protection Agency 1982 and 1983; and Schneider and Kaplan 1983

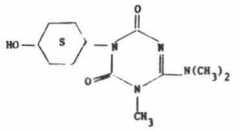
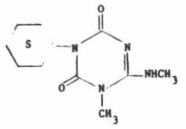
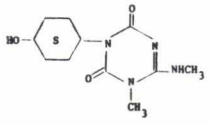
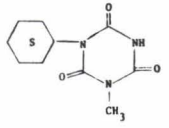
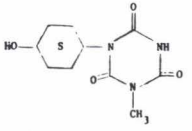
Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity		
Hexazinone (90% soluble powder)	Rat (male female, ChR:CD)	Fed 0, 200, 1,000, 2,500, and 10,000 ppm in diet to weanlings for 2 years	NOEL = 200 ppm; no carcinogenic effects	--	Newton and Dost 1981 and Ghassemi et al. 1981
Hexazinone (unformulated)	Mice (male and female, weanling, Crl:CD-1)	Fed 0, 200, 2,500, and 10,000 ppm in diet for 2 years to 80 mice per sex per group. Hemotology at 6 intervals from 1 to 24 months.	NOEL = 200 ppm; no evidence for compound-related carcinogenicity. Non-neoplastic hepato-cellular effects observed in both sexes at 10,000 ppm and in males at 2,500 ppm.	--	U.S. Environmental Protection Agency 1983 and Schneider and Kaplan 1983

[a]Data not available or not applicable.
[b]Crl:CD and ChR:CD are designations used for same strain of Charles River rat.

Appendix D-1

Summary of Acute Oral Toxicity of Metabolites

Metabolite		Organism	Nature of Exposure	Effects
Metabolite A [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1 methyl-1,3,5-triazine-2,4(1H,3H)-dione]		Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 1 animal per dose level. Dose levels not specified. Animals weighed and observed for 14 days.	ALD >4,686 mg/kg body weight; for 1 day after dosing at 2,250 and 4,686 ppm observed weight loss; diarrhea observed on day after dosing at 2,250 ppm; wet perinea observed on day after dosing at 4,686 ppm.
Metabolite B [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2-4(1H,3H)-dione]		Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 1 animal per dose level. Dose levels not specified. Animals weighed and observed for 14 days.	ALD > 5,000 mg/kg body weight; observed weight loss for 1 to 2 days after dosing; slightly stained perinea observed 2 to 4 days after dosing at 5,000 mg/kg.
Metabolite C [3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione]		Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 1 animal per dose level. Dose levels not specified. Animals weighed and observed for 14 days.	ALD >7,500 mg/kg body weight; observed weight loss in sporadic groups from 1,000 mg/kg and higher doses; on day after dosing observed congestion at 2,250 ppm and wet perinea at 5,000 ppm.
Metabolite D [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione]		Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 1 animal per dose level. Dose levels not specified. Animals weighed and observed for 14 days.	ALD >7,500 mg/kg body weight; observed weight loss 1 to 3 days after dosing and chromodacryorrhea at 2,250 ppm, increased respiration at 3,400 and 5,000 ppm, congestion at 3,400, 5,000, and 7,500 ppm; fasciculations at 5,000 and 7,500 ppm and diarrhea, stained nose, and wet and stained perinea at 7,500 ppm.
Metabolite E [3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione]		Rat (male, Crl:CD)	Single dose of corn oil suspension administered by intubation to 1 animal per dose level. Dose levels not specified. Animals weighed and observed for 14 days.	ALD >7,500 mg/kg body weight; observed weight loss, wet and stained perinea, salivation, diarrhea, rapid breathing, and chromodacryorrhea.

Source: Schneider and Kaplan 1983.

Appendix E

Summary of Hexazinone Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	REFRENCES
Hexazinone (unformulated)	Salmonella typhimurium 5 strains, unspecified	Grew tester strains in media containing doses of 400, 800, 1,200, 1,600, and 2,000 g/plate of test material both in the presence and absence of activation system.	Nonmutagenic	--[a]	Newton and Dost 1981; Ghassemi et al. 1981; and U.S. Environmental Protection Agency 1982 and 1983
Hexazinone (unformulated)	Chinese Hamster Ovary (CHO) cells; point mutation assay	Incubated CHO cells in treatment media in the presence and absence of activation system. Cells subcultured to allow growth and expression of cytotoxicity, then seeded in media with and without selective agents to allow enumeration of HGPRT deficient mutants.	Nonmutagenic	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Chinese Hamster Ovary (CHO) cell in vitro cytogenetic assay	Exposed CHO cells to test material concentrations including 2, 4, and 5 mg/ml in the presence and absence of metabolic activation system. Cells then treated with colcemid to arrest cells in metaphase. Cells harvested for preparation and scoring of chromosome profile.	Chromosome damage induced under non-activation conditions at test concentrations of 4 and 5 mg/ml but not at 2 mg/ml or lower. Test material clastogenic under activation conditions in one of two assays with 4 mg/ml.	--	Schneider and Kaplan 1983

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Hexazinone (unformulated)	Rat (Sprague-Drawley) *in vivo* bone marrow cytogenetic assay	Applied single doses of 0, 100, 300, or 1,000 mg test material/kg body weight to groups of 12 male and 12 female rats. Administered colchicine to 3 rats per sex 6, 12, 24, and 48 hours after test material to arrest cells in metaphase. Rats were sacrificed. Bone marrow cells were collected from the femurs, prepared, and examined.	No test material-related chromosomal aberrations detected. Five deaths at 1,000 mg/kg. Toxic signs observed were from slight depression at 300 mg/kg to convulsions at 1,000 mg/kg.	--	Schneider and Kaplan 1983
Hexazinone (unformulated)	Rat hepatocytes; *in vitro*-unscheduled DNA synthesis	Treated freshly isolated hepatocytes with test material followed by radiolabeled thymidine. Dose not specified. Quantitated thymidine incorporation into DNA autoradiographically.	Observed no induction of unscheduled DNA synthesis.	--	Schneider and Kaplan 1983

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Hexazinone

Formulations	Organisms	Dose	Comments	References
Hexazinone (^{14}C)	Rat (single male)	Single oral dose administered by intubation to a rat pre-treated for 17 days with unlabeled hexazinone in diet at 2,500 ppm.	Radioactivity eliminated within 72 hours, mostly within 24 hours; 61% in urine, 32% in feces. Trace amounts in gut and expired air, none in body tissues (<0.01% of original (^{14}C).	Rhodes and Jewell 1980
Hexazinone (^{14}C)	Rat (males and females)	Animals were variously dosed as follows: A. A single treatment of 14 mg per kg without preconditioning. B. A single treatment of 14 mg per kg following 3 weeks of 100 ppm dietary unlabeled hexazinone. C. A single treatment of 1,000 mg per kg without preconditioning.	Labeled material was rapidly excreted in urine and feces as follows: A. 14 mg/kg non-preconditioned rats eliminated 67% labeled material in first 24 hours, 94.2% by 72 hours. B. 14 mg/kg preconditioned rats eliminated 88% labeled material in first 24 hours, 100% by 72 hours. C. 1,000 mg/kg non-preconditioned rats eliminated 88% labeled material in first 48 hours, 96% by 144 hours. Only trace residues in gut, skin, muscles, and viscera.	Schneider and Kaplan 1983
Hexazinone (^{14}C)	Goat (female)	Five daily doses at 1 or 5 ppm in diet of 2 lactating goats.	Hexazinone rapidly eliminated in urine (80%) and feces (6%). Milk residues plateaued on day 2 at 0.02 and 0.05 ppm for 1 and 5 ppm doses respectively. Animals sacrificed 1 day after last dose - meat and fat of 5 ppm dosed animal with 0.01 ppm residues, liver residues 0.09 ppm (primarily unmetabolized hexazinone), kidney residues 0.04 ppm.	Schneider and Kaplan 1983

Appendix F (Concluded)

Formulations	Organisms	Dose	Comments	References
Hexazinone (90% soluble powder)	Dairy Cow (female)	30 daily doses at 0, 1, 5, and 25 ppm a.i. administered in diet to 2 cows per dose. One cow per group sacrified on day 31, the second cow per group sacrified after an additional 7 days on control diet.	Trace levels (maximum 0.05 ppm) of metabolite B in milk at 31 days in cow fed 25 ppm but none after 3 days withdrawal. No hexazinone or metabolites detected in muscle, fat, liver, or kidney at any dose tested. No gross clinical or pathological effects.	Schneider and Kaplan 1983
Hexazinone	Chicken (female, leghorn)	Dosed for 4 weeks at 0, 1, and 5 ppm in diet.	No adverse effects on body weight, egg production or health observed. No residues of hexazinone or metabolites detected in eggs, skin, muscle, fat or liver.	Schneider and Kaplan 1983
Hexazinone	Bluegill	Fish exposed under dynamic conditions to 0.01 and 1.0 ppm for 4 weeks followed by 2 weeks in water without hexazinone.	^{14}-C residues plateaued after 1 to 2 week exposure. Maximum residues for 0.01 ppm exposure were: carcass, 0.02 ppm; liver, 0.03 ppm; and viscera, 0.07 ppm. Maximum residues for 1.0 ppm exposure were: carcass, 2.1 ppm; liver, 5.0 ppm; and viscera, 6.7 ppm. After 1 week in clean water, radiolabeled residues decreased by more than 90% and after 2 weeks in clean water, no ^{14}C residues could be detected. No adverse effects were noted.	Rhodes 1980b

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Herbicide Background Statement: Picloram

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Picloram Summary

Major Trade Names: Tordon®, Amdon®

Chemical Name: 4-amino-3,5,6-trichloropicolinic acid

Major Forestry Applications: Range management and noxious weed control, site preparation, pre-commercial thinning, and conifer release.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Picloram and its salts are low in toxicity to most nontarget organisms. Picloram is relatively nontoxic to soil microorganisms at concentrations up to 1,000 ppm. For most species of fish, picloram formulations are only slightly toxic with median lethal concentrations of greater than 10 ppm. The acute toxicity for birds is greater than 2,000 mg/kg. In subchronic feeding studies, with birds, the LC_{50} is greater than 5,000 ppm.

In studies with experimental and farm animals, the acute toxicity ranged from 8,200 mg/kg in rats to greater than 950 mg/kg in cattle. Tests with rabbits indicate that picloram is not likely to be absorbed through the skin. The LD_{50} in dermal toxicity tests with rabbits is greater than 4,000 mg/kg. Although it is a mild skin irritant in rabbits, patch tests show that it does not sensitize the skin of humans. Rats, exposed to a saturated atmosphere of picloram formulation for 7 hours, showed no significant adverse effects indicating that inhalation of picloram is not likely to cause illness. Since picloram induces only moderate eye irritation in rabbits, which heals readily, it is not likely to cause injury to the cornea or blindness. Long-term studies in rats and dogs showed no observable adverse effects when doses of up to 150 mg picloram/kg body weight were fed for 2 years. Studies in rats and mice showed that picloram is nonteratogenic even at doses toxic to the pregnant animals, and has little or no effect on fertility, reproduction, or development of offspring. Picloram was generally found to be nonmutagenic and noncytogenetic. It appears to present little or no carcinogenic risk, although bioassays on mice and rats suggested the ability to induce benign liver tumors in rats.

Environmental Fate

Picloram is readily absorbed by plant roots and translocated throughout plant tissues. It is particularly prone to accumulate in new plant growth, where it is quite stable and remains largely intact. In animals, picloram does not bioaccumulate. Picloram that is ingested by animals is rapidly excreted unchanged, primarily in the urine.

Picloram is moderately to highly persistent in soil, with a half-life of approximately one month under highly favorable conditions of moisture, temperature, and organic conditions and a half-life of more than 4 years in arid regions. It is a relatively mobile herbicide. The potassium salt and acid formulations of picloram are easily leached from the soil. Picloram, especially in salt formulations, is water soluble and thus has a potential for high concentration in runoff from heavy rainfall occurring soon after applications. Under such conditions, concentrations of 400 to 800 ppb have been detected. However, studies have indicated that runoff accounts for less than 3 percent of the total quantity of picloram applied to soil.

Exposure and Hazard

Estimated occupational environmental exposures to picloram from normal use are well below those levels that would be expected to have acute or chronic toxic effects or reproductive effects. Picloram has not been shown to be mutagenic or carcinogenic at exposures well in excess of those to which forest workers, visitors, and animal residents would be exposed.

1.0 General Information

Picloram, also known by the trade names* Tordon® and Amdon® is a wide-spectrum herbicide which can be used to effectively control a variety of woody plants and annual and perennial broadleaved weeds (Weed Science Society of America 1983 and Meister Publishing Co. 1981). Although the precise mode of herbicidal action of picloram has not been determined, it is known to function as a plant growth regulator. Picloram is absorbed by plant roots and foliage, is translocated throughout the plant, and is accumulated at sites of new growth (Kearney and Kaufman 1975 and Witt and Baumgartner 1979).

1.1 Normal Use Patterns

Picloram is used in a variety of vegetative management programs administered by the USDA Forest Service as well as by state and local agencies. Most of it is used for site preparation, conifer release, timber stand improvement, and control of noxious weeds. A survey of picloram use by the USDA Forest Service in 1981 and 1982 for various methods of application is shown in Table 1-1. Picloram formulations used in forestry applications include the highly water soluble amine and potassium salts of the compound. Table 1-2 indicates the percentage of active ingredient as well as the inert carriers used in these formulations (Ghassemi et al. 1981).

In forestry applications, solutions of liquid formulations are sprayed on foliage, injected into plants, and applied to cut surfaces of plants. Picloram pellets are applied to plant bases where the active ingredient is leached to plant root zones (Schlapfer 1977). Liquid formulations are usually aqueous solutions applied at rates of 2 ozs. to 3 lb picloram/acre, or 1 ml per injection using Tordon 101® or 101R®.

The granular formulation is applied by granular applicators or by hand at the rate of 2 to 8 lb picloram/acre. Picloram applications are usually made from spring of the year to 3 weeks before the first frost (Weed Science Society of America 1983; National Forest Products Association 1980; Ghassemi et al. 1981).

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service Use of Picloram

Purpose	Acid Equivalent Per Acre (lbs)		Acres Treated	Formulation[a]	Application Method
	Range	Average			
	1981				
Aerial					
Right-of-way	1.9	1.9	353	Liquid	Helicopter
Site preparation	0.75	0.75	1,244	Liquid	Helicopter
Ground					
Noxious weeds	0.3 - 5.0	3.0	6,157	16% Liquid 84% Pellet	Broadcast
Poisonous plants	0.2 - 2.0	1.3	351	50% Liquid 50% Pellet	Individual plant, Broadcast
Range improvement	0.3 - 1.5	0.6	4,205	1% Liquid 99% Pellet	Broadcast
Right-of-way	0.2 - 1.0	0.5	429	90% Liquid 10% Pellet	Basal stem, Cut stump, Broadcast
Conifer release	0.1 - 0.5	0.4	19,304	100% Liquid	Injection, Cut stump, Broadcast
Firebreak management	2.0	2.0	318	100% Pellet	Broadcast
Site preparation	0.3 - 0.7	0.5	41,381	100% Liquid	Broadcast
Precommercial thinning	0.2	0.2	2,982	100% Liquid	Injection
Wildlife habitat improvement	0.2 - 1.5	1.0	6,588	54% Pellet 46% Liquid	Injection, Cut stump, Broadcast

Table 1-1 (Concluded)

Purpose	Acid Equivalent Per Acre (lbs)		Acres Treated	Formulation[a]	Application Method
	Range	Average			
	1982				
Aerial					
Timber	1.0	1.0	--[b]	Tordon K®	Aerial
Noxious weeds	--	--	--	Tordon 22K®	Aerial
Timber, Right-of-way	1.0	1.0	--	Tordon 101®	Aerial
Ground					
Range, Noxious weeds	0.3 - 2.0	1.3	--	Beads	Hand Broadcast
Range, Noxious weeds	0.25 - 2.0	1.6	--	Tordon 2K®	Hand Broadcast
Wildlife, Range, Timber, Fuels Management	0.22 - 14.0	3.1	--	Tordon 10K®	Hand Broadcast
Range, Noxious Weeds, Right-of-way	0.25 - 5.0	1.4	--	Tordon 22K®	Ground Foliar
Timber, Recreation	0.25 - 2.0	0.7	--	Tordon 101®	Injection, Cut stump
Timber, Range, Noxious Weeds	0.5 - 4.0	1.6	--	Tordon 101®	Ground Foliar
Timber, Wildlife	0.1 - 0.5	0.2	--	Tordon 101R®	Injection, Cut Stump
Range, Noxious Weeds	2.5 - 10.0	5.3	--	Tordon 212®	Ground Foliar
			Total 59,806		

[a]Liquid formulation is a 4:1 mixture of 2,4-D to picloram. Tordon 101® formulations are a 4:1 mixture of 2,4-D to picloram; Tordon 212® is a 2:1 mixture of 2,4-D to picloram.

[b]Specific data not available.

Source: Gross 1983.

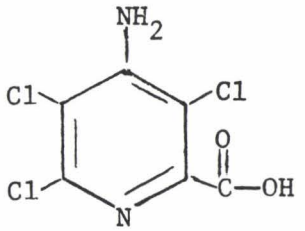
Table 1-2

Picloram Formulations for Forestry Use

Formulation[a]	Active Ingredients (Percent)	Active Ingredients (Weight)	Inert Carriers
Tordon Beads®	2.3% picloram-potassium salt (2.0% acid equivalent, a.e.)	0.32 oz a.e./lb as the potassium salt	No inert carriers since active ingredients in salt form are 98% of formulation.
	79.2% disodium tetraborate pentahydrate (DSTP)	1.9 oz/lb boron from DSTP	
	16.5% disodium tetraborate decahydrate (DSTD) (43.8% a.e. boron trioxide)	0.29 oz/lb boron from DSTD	
Tordon K®	24.0% picloram-potassium salt (20.8% a.e.)	2.0 lb a.e./gal as the potassium salt	Not given
Tordon 10K®	11.6% picloram-potassium salt (10% a.e.)	1.6 oz a.e./lb as the potassium salt	Clay
Tordon 22K®	24.4% picloram-potassium salt (21.1% a.e.)	2 lb a.e./gal as the potassium salt	The mixture also contains glycol and sorbital ester type wetting agents along with alcohol and water.
Tordon 101® Mixture	10.2% picloram-triisopropanolamine salt (5.7% a.e.) 39.6% 2,4-D-triisopropanolamine salt.	10 oz a.e./gal as the triisopropanolamine salt. 40 oz a.e./gal 2,4-D-triisopropanolamine salt	The mixture also contains a glycol derivative sequestrant and glycol wetting agent along with alcohol and water.
Tordon 101R® Mixture	5.4% picloram-triisopropanolamine salt. 20.9% 2,4-D-triisopropanolamine salt.	4.2 oz a.e./gal as the triisopropanolamine salt. 16.7 oz a.e./gal. as the 2,4-D-triisopropanolamine salt	The mixture also contains a glycol derivative sequestrant and glycol wetting agent along with alcohol and water.
Tordon 155® Mixture	15.1% picloram-isooctyl ester (10.3% a.e.)	1 lb a.e./gal as the isooctyl ester	The mixture also contains a petroleum solvent and alcohol.
	63.4% 2,4,5-T-propylene glycol butyl ether (PGBE) esters (41.3% a.e.)	4 lbs a.e./gal as the 2,4,5-T PGBE esters	
Tordon 212® Mixture	18.1% picloram as the triisopropanolamine salt (10.1% a.e.)	1 lb a.e./gal as the salt	The mixture also contains a glycol derivative sequestrant and glycol wetting agent along with alcohol and water.
	37.7% 2,4-D-triisopropanolamine salt (20.2% a.e.)	2 lb a.e./gal as the salt	

1.2 Physical and Chemical Properties

The active ingredient of picloram is 4-amino-3,5,6-trichloropicolinic acid. It has a molecular formula of $C_6H_3Cl_3N_2O_2$ and a molecular weight of 241.48. The structural formula is given below (Weed Science Society of America 1983):



Picloram is a white colorless crystalline substance which decomposes at 215°C before melting at 218-219°C. It is stable in both acidic and basic solutions. Picloram is only very slightly soluble in water (430 ppm), but its potassium and amine salts are highly water soluble (Kearney and Kaufman 1975; Melnikov 1971 and Ramsey 1967).

2.0 Toxicity of Picloram

A summary of the toxicological properties of picloram (Table 2-1) shows that this herbicide has low toxicity to most organisms. Studies with rodents indicate that picloram is nonteratogenic; has little or no effect on fertility, reproduction, or development of offspring; is nonmutagenic; and appears to present little or no carcinogenic risk.

2.1 Invertebrates and Microorganisms

A variety of invertebrates and microorganisms from freshwater, marine, and terrestrial ecosystems have been studied for toxic effects of picloram and picloram formulations. Major findings of these studies are presented in Appendix A and indicate a low toxicity of picloram, although there is a broad range of susceptibility to picloram in the organisms tested.

For the most part, toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results of such studies do not necessarily present a true reflection of the potential effects of picloram in a natural environment. For example, in the laboratory, aquatic organisms are kept in water containing dissolved picloram at various concentrations and microorganisms are grown in a culture medium containing picloram. In many instances, the exposures are maintained for days, weeks, or even months. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to picloram or other man-made chemicals is usually a transient phenomenon depending on streamflow, chemical dissipation and decomposition. Additionally, in natural systems, interactions with other man-made or naturally occurring chemicals, as well as changes in environmental factors such as temperature and moisture, all potentially affect herbicide assimilation and metabolism by invertebrates and microorganisms and thus alter their tolerance levels.

Among the insects tested (Appendix A), honeybees, representing insects of commercial value, showed no observable effect and no increase in mortality when sprayed with or fed picloram at 1,000 ppm in a 60 percent sucrose syrup. Furthermore, caged honeybees sprayed with Tordon 212® and Tordon 22K®-water mixture at a rate of 4 lb active ingredient (a.i.) per acre (4.48 kg/ha) (roughly equivalent to the maximum normal application rate of aqueous solutions of picloram formulations) showed no increase over normal mortality rates 14 days after spraying. Most aquatic arthropods (crustaceans and insects) exposed for 24 hours to picloram had LC_{50}'s ranging from 50 to 120 ppm. Daphnia, however, showed no observable effect at 380 ppm picloram during a 24-hour exposure period.

Table 2-1

Summary of Toxicological Properties of Picloram

1. Acute Oral Toxicity

Organism	LD_{50} (mg/kg)
Rats	8,200
Mice	2,000 to 4,000
Rabbits	Approximately 2,000
Guinea Pigs	Approximately 3,000
Chicks	Approximately 6,000
Sheep	Greater than 1,000
Cattle	Greater than 750
Birds	Greater than 2,000
Fish	LC_{50} generally greater than 10 ppm in water

2. Subchronic and Chronic Oral Toxicity

Organism	Length of Treatment	Dose Tested	Adverse Effects
Sheep	30 days	100 mg/kg	None
Sheep	10 days	250 mg/kg	Lethal (8/10)
Calves	31 days	15.4 mg/kg	None
Rats	90 days	1,000 ppm	None
Rats	90 days	3,000 ppm	Liver damage
Rats	2 years	150 mg/kg	None
Dogs	2 years	150 mg/kg	None

3. Dermal, Eye, and Inhalation Toxicity

Organism	Dose Tested	Adverse Effects
Rabbit	Up to 4,000 mg/kg	Skin - LD_{50} from skin absorption is greater than 4,000 mg/kg. Mild skin irritant.
Rabbit	Undiluted or 10% slurry	Eyes - moderate eye irritation which heals readily. No corneal damage or blindness.
Rat	Saturated atmosphere	Inhalation - no adverse effects.

Table 2-1 (Continued)

4. Teratology, Reproduction, and Fertility

Organism	Length of Treatment	Adverse Effects
Rat	150 mg/kg/day for 3 generations	None
Rat	Up to 1,000 mg/kg/day from days 6 to 15 of gestation	Only minor abnormalities seen at 750 or 1,000 mg/kg but no teratogenesis or adverse effects on neonatal development.
Mouse	15 mg/kg/day from 4 days before to 14 days after mating	No effect on fertility or number of offspring.

5. Mutagenicity and Cytogenicity

Organism	Adverse Effects
Salmonella typhimurium; Escherichia coli T_4 bacteriophage; Aspergillus nidulans	No evidence of mutagenicity.
Streptomyces coelicolor	Increase in revertant colonies. System not validated.
Saccharomyces cerevisiae	Increase (not statistically significant) in revertants with one strain tested.
Rat	No evidence of cytogenetic effects on bone marrow cells when fed up to 2,000 mg/kg.

Table 2-1 (Concluded)

6. Carcinogenicity

Organism	Length of Treatment	Adverse Effects
Dog	150 mg/kg/day for 2 years	Noncarcinogenic.
Rat	150 mg/kg/day for 2 years	Tumor incidence comparable to controls.
Rat	14,875 and 7,431 ppm in diet for 80 weeks	Not carcinogenic in male; benign liver nodules in females.
Mouse	5,062 and 2,531 ppm in diet for 80 weeks	Not carcinogenic in male or female animals.

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

Picloram is considered to be relatively nontoxic to soil microorganisms (National Research Council of Canada 1974). Fungi appear to be generally able to tolerate concentrations of picloram as high as 1,000 ppm. At moderate concentrations (up to 10 ppm), some species exhibited stimulated growth rates.

2.2 Fish

A large number of studies have addressed the potential toxicity of picloram formulations to fish species. Early studies were reviewed extensively by Kenaga (1969) and the results of these studies, as well as more recent ones, are summarized in Appendix B. Most formulations of picloram are, at worst, only slightly toxic (Clarke et al. 1970) to most fish species with an $LC_{50} > 10$ ppm. Exceptions noted in Appendix B include Tordon 22K® toxicity to bluegill (LC_{50} of 5.4 to 8.2 ppm), and Technical Grade picloram (90% a.i.) toxicity to cutthroat trout and lake trout (LC_{50} of 5.0 ppm and 4.25 ppm, respectively) for 96-hour bioassay exposures.

2.3 Birds

The toxic effects of picloram in birds have been investigated in a small number of studies summarized in Appendix C. In general, picloram is relatively nontoxic to birds.

Studies of acute toxicity with technical grade (90.5% a.i.) formulations fed in single doses to male mallard ducks and pheasants resulted in LD_{50}'s of greater than 2,000 mg/kg. Similar feeding studies of Tordon 22K® with mallards resulted in the same LD_{50} levels.

Several feeding studies in birds have been conducted with picloram. For the most part, when birds were fed picloram in the diet for 5 days, the LC_{50}'s were greater than 5,000 ppm. Japanese quail fed 1,000 mg picloram per kg body weight for two weeks experienced a decrease in egg fertility and hatchability.

A series of studies have been conducted on the effects of Tordon 22K® and a 4:1 mixture of 2,4-D: picloram on reproduction in chickens. Eggs were exposed to application rates equivalent to as much as 11.2 kg a.i./ha (10 lb per acre) for as long as three days and showed no observable effects.

2.4 Mammals

The potential health hazards from exposure to picloram have been evaluated in various species of mammals. Studies have been done mostly with laboratory animals such as mice, rats, guinea pigs, and

rabbits. Limited studies were done on dogs and on farm animals such as sheep, pigs, calves, dairy cows, and steers. No studies have been conducted on wildlife to date.

Results of the various toxicity tests are described below and summarized in Table 2-1 and Appendix D.

2.4.1 Acute Toxicity

The data on acute toxicity were summarized from Lynn (1965), unless otherwise specified.

2.4.1.1 Oral. Picloram has low acute toxicity in a number of animal species. The acute oral LD_{50} in the rat is 8,200 mg/kg. In the mouse, rabbit, and guinea pig, the acute oral LD_{50} is 2,000-4,000, 2,000, and 3,000 mg/kg, respectively. Sheep showed no ill effects when given one dose of picloram up to 650 mg/kg or Tordon 22K® (25% a.i.) potassium salt formulation up to 4,650 mg/kg. Cattle showed no ill effects after one oral treatment of up to 488 mg/kg picloram or up to 3,480 mg/kg Tordon 22K®. The acute oral LD_{50} for the isooctyl ester of picloram is 3,250 mg/kg (a 5 percent confidence limit of 2,480 to 4,260 mg/kg) in male rats and 2,830 mg/kg in female rats (Dow Chemical Company 1984).

2.4.1.2 Dermal. Tests in rabbits show that picloram is not likely to be absorbed through the skin. It is a mild skin irritant in rabbits. The shaved skin of albino rabbits was exposed to graded doses of picloram (up to 4 g/kg) or Tordon 22K® (up to 2 g/kg) for 24 hours. No mortality or toxic signs were observed. The exposed area of the skin of rabbits treated with picloram, but not Tordon 22K®, showed slight edema (swelling) and brown discoloration. The acute dermal LD_{50} for the isooctyl ester of picloram is 2,000 mg/kg in male rabbits (Dow Chemical Company 1984).

2.4.1.3 Inhalation. Inhalation of picloram is not likely to cause illness. To test for inhalation effects, albino rats were exposed for 7 hours to an atmosphere saturated with Tordon 22K®. No adverse effects were observed during the period of exposure or for the following 2 weeks.

2.4.1.4 Primary Eye Irritation. Picloram induces moderate eye irritation in rabbits which heals readily, indicating that it is not likely to cause injury to the cornea or blindness. When undiluted or a 10 percent slurry of picloram was applied directly to the conjunctival sac of the eyes of albino rabbits, the material was only slightly irritating and produced a slight conjunctivitis which subsided in one to two days. Rabbits, similarly treated with undiluted Tordon 22K®, exhibited only slight conjunctival redness and

very slight corneal cloudiness which disappeared in 24 to 48 hours. The isooctyl ester of picloram (Dow Chemical Company 1984) was essentially nonirritating to either washed or unwashed eyes of one rabbit.

2.4.2 Subchronic Toxicity

2.4.2.1 Oral. Generally little or no effects on test animals were observed after subchronic exposure to picloram in the diet. No toxic effects were observed in rats fed up to 1,000 ppm picloram in the diet (equivalent to 75 mg/kg body weight) for 90 days (Lynn 1965). Moderate histological changes in the liver and kidneys and slight loss of body weight were observed in rats treated with 3,000 and 10,000 ppm picloram (equivalent to 225 and 750 mg/kg body weight, respectively). No adverse effects were noted in rats fed up to 3,000 ppm (equivalent to 225 mg/kg body weight) of the triisopropanolamine salt of picloram in the diet. No toxic signs or adverse effects on growth were noted when yearling sheep were fed 18 mg/kg body weight (equivalent to 450 ppm) of picloram in the diet for 33 days. Sheep fed 10 doses of the potassium salt of picloram at 100 mg/kg body weight (2,500 ppm) showed no toxic effects, although 8 of 10 sheep died after 10 doses at 250 mg/kg (Palmer and Radeleff 1969). Sheep fed up to 100 mg/kg body weight acid equivalent of Tordon 22K® for 30 days showed no toxic signs or adverse effects on growth (Jackson 1965 and Lynn 1965). Calves fed up to 15.4 mg/kg body weight of picloram in the diet for 31 days showed no toxic effects. Interestingly, stimulated growth and improved feed efficiency was observed in swine fed 22 mg of picloram/kg feed which is equivalent to 0.88 mg/kg body weight (McCollister and Leng 1969).

More recent subchronic oral toxicity studies in rats, dogs, and mice have been reported (Dow Chemical Company 1984). In the study on CDF Fischer 344 rats, 15 animals per sex per dose were fed 0, 15, 50, 150, 300, and 500 mg technical grade picloram per kg body weight per day for 13 weeks. According to the summarized results, no deaths were observed. Mean body weights and food consumption were comparable to the controls or not dose related. Clinical chemistry determinations made on all rats at the termination of the study revealed no significant differences from control values. Hematologic determinations and urinalyses made on 10 rats per sex per dose during the 12th week of the study and differential leukocyte counts made on 10 rats per sex of the highest dose and control groups revealed no significant differences from the controls.

A dose-dependent increase in the absolute and relative liver weights occurred in rats given 150, 300, or 500 mg/kg/day. Male rats given 300 and 500 mg/kg/day also exhibited increased absolute and relative kidney weights.

Microscopic examination of a variety of tissues collected from 10 rats per sex of the control and high level (500 mg/kg/day) groups and sections of liver and kidneys from 10 rats per sex in the other treatment groups revealed minimal changes in the liver of animals of both sexes given 150, 300, or 500 mg/kg/day. The subchronic NOEL for rats is 50 mg/kg/day.

Beagle dogs (6/sex/dose) were fed 0, 7, 35, or 175 mg technical grade picloram/kg/day for six months (Dow Chemical Company 1984). A number of treatment related adverse effects were reported. Animals of both sexes treated with the highest dose of picloram (175 mg/kg) exhibited decreased body weight, body weight gain, food consumption, and alanine transaminase levels, and increased alkaline phosphatase level, absolute and relative liver weights. Absolute and relative liver weights were also observed in males given 35 mg/kg. The liver was the primary target organ of picloram. No treatment-related effects were detected in females of the 35 mg/kg group or in males or females of the lowest (7 mg/kg) dose level groups. The subchronic NOEL for dogs therefore was 7 mg/kg body weight/day.

A 13-week feeding study has been conducted in B6C3F1 mice (Dow Chemical Company 1984). Groups of 10 mice per sex per dose were fed diets to provide 0, 1,000, 1,400, or 2,000 mg picloram per kg body weight per day for 13 weeks. Clinical observations, food consumption, urinalyses, and hematologic determinations revealed no significant differences from the controls. Terminal body weights of female mice fed 2,000 mg/kg were reduced compared with controls. Serum alkaline phosphatase levels were reduced to varying degrees in all groups of mice. Absolute and relative liver weights were increased in females at all dose levels and in males at 1,000, 1,400, and 2,000 mg/kg/day. Microscopic examination of a wide range of organs and tissues showed dose related morphologic alterations of hepatocytes in female mice at all doses and in all males given 1,400 or 2,000 mg/kg/day. Hepatic alterations were detected in most male mice given picloram at 1,000 mg/kg/day. A NOEL was not determined.

In a second mouse study (Dow Chemical Company 1984), groups of six mice per sex were fed a diet which provided 0, 30, 100, 650, 1,000, or 3,000 mg picloram/kg body weight/day for 32 days. In this 32-day tolerance study, a dose of 3,000 mg picloram/kg/day had effects on the livers and gastric mucosa. No adverse effects were reported for mice receiving 1,000 mg or less picloram/kg/day.

2.4.2.2 Dermal Irritation. Repeated exposure of the shaved, abraded, or intact skin of albino rabbits with undiluted picloram daily for 9 times over a period of 11 days resulted only in slight exfoliation (scaling of the skin) and hyperemia (congestion and redness) at the exposure site. When the skin of rabbits was repeatedly exposed to a cotton pad saturated with Tordon 22K® for 10

days over a 2-week period, slight hyperemia and edema, with eschar (scab) formation in one animal, occurred in the treated areas. However, the skin returned to normal 21 days after treatment (Lynn 1965). Slight redness, slight swelling, and slight superficial necrosis occurred during the period of 10 applications of the isooctyl ester of picloram to the intact or abraded skin of one rabbit. Necrosis occurred sooner when test material was applied to abraded skin (Dow Chemical Company 1984).

Repeated dermal applications of a 10 percent solution of Tordon 22K® were made on human subjects without any skin irritation or skin sensitization attributable to exposure to the formulation (Lynn 1965). It has been stated (Dow Chemical Company 1984) that a number of picloram formulations (6 percent Tordon® acid + 22 percent 2,4-D acid; 10.2 percent picloram TIDA salt + 39.6 percent 2,4-D TIDA salt; and 10 percent picloram salt) have been tested without effects for dermal irritancy and skin sensitization potential in humans. No supporting data were provided.

2.4.3 Chronic Toxicity

Picloram in the diet was not toxic to dogs and rats. In long-term feeding studies, beagle dogs and albino rats were fed picloram at doses of 15 to 150 mg/kg of body weight for 2 years (Lynn 1965 and McCollister and Leng 1969). No observable toxic effects were noted in either species in terms of body weight, food consumption, mortality, behavior, hematology, clinical blood chemistry or urine chemistry. These studies were conducted by a commercial laboratory under contract to Dow. The results cannot be validated because the raw data has been destroyed.

2.4.4 Reproductive and Teratogenic Effects

Picloram appears to have little or no effect on fertility, reproduction or development of offspring. In a multigeneration reproduction study, rats were fed with up to 0.3 percent picloram in the diet (equivalent to 150 mg/kg body weight) for three generations. No significant differences between treated and untreated animals were observed regarding gestation, viability, lactation, weanling weights, rates of resorption of fetuses, or fetal teratogenicity (Copeland et al. 1967; McCollister and Leng 1969; and Dow Chemical Company 1984).

In a teratology study in Sprague-Dawley rats, picloram was fed at dose levels up to 1,000 mg/kg body weight from the 6th through the 15th day of gestation. No adverse effects on fetal development were observed at doses of 500 or 750 mg/kg/day. The endpoints measured included gestation period, number of embryos implanted and resorbed,

number of corpora lutea (hormone-secreting structures in the female ovary), litter size, fetal weight, pup weight, sex ratio, viability, and lactation. Some minor nonteratogenic abnormalities were seen in animals treated at doses of 750 or 1,000 mg/kg/day. An increase in unossified fifth sternebrae reflecting retarded fetal growth was observed in the treated groups. The authors concluded that oral administration of subtoxic or even maternally toxic doses of picloram during organogenesis produced neither teratogenesis nor adverse effects on early development of the newborn rat (Thompson et al. 1972).

As part of a three-generation reproduction study, 12 female rats at each generation were fed dietary levels of 0.03, 0.1, or 0.3 percent picloram (Dow Chemical Company 1984). Doses were not high enough to cause maternal toxicity. Maternal rats were sacrificed on day 19 or 20 of gestation and the fetuses were examined for gross abnormalities and placentas were examined for fetal death or resorptions. No teratological effects were observed in the groups receiving up to 0.3 percent picloram.

In an antifertility study in which mice were fed 100 ppm (or 15 mg/kg body weight) picloram in the diet for four days before and for 14 days after mating, there were no adverse effects on fertility or the number of offspring produced (Hayden et al. 1963 and McCollister and Leng 1969).

2.4.5 Carcinogenicity

Picloram appears to present little or no carcinogenic risk to man. In long-term feeding studies, groups of albino rats and beagle dogs were fed up to 150 mg/kg body weight of picloram in their diets for two years. Gross and histopathological examinations of the tissues showed tumor incidence to be comparable in treated and untreated control rats. No tumors were induced in the dogs (Lynn 1965 and McCollister and Leng 1969). These studies were conducted by a commercial testing laboratory under contract to Dow and have not been validated because the raw data have been destroyed. A two-year chronic/oncogenicity study of picloram in rats was initiated in 1982 (Dow Chemical Company 1984).

A carcinogenesis bioassay of picloram in rats and mice was conducted by Gulf Research Institute for the National Cancer Institute (NCI) (National Cancer Institute 1978). Fifty Osborne-Mendel rats and 50 B6C3F1 mice of both sexes were fed picloram at time-weighted dietary levels of 7,437 or 14,875 ppm (approximately 380 or 750 mg/kg/day) for rats and 2,531 or 5,062 ppm (approximately 380 or 760 mg/kg/day) for mice for 80 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex. Pooled

controls, used for statistical evaluation, consisted of matched-control groups combined with 30 untreated male and 30 untreated female rats or mice from similar bioassays of three other test chemicals. The rats and mice were then observed for an additional 33 and 10 weeks, respectively, at which time all surviving animals were sacrificed.

In rats, a relatively high incidence of follicular hyperplasia, C-cell hyperplasia, and C-cell adenoma of the thyroid occurred in both sexes. However, the statistical tests for adenoma did not show sufficient evidence for association of the tumor with picloram administration.

An increased incidence, as compared with untreated animals, of hepatic neoplastic nodules (considered to be a benign tumor) was observed in treated rats of both sexes. In male rats, the lesion appeared only in three animals treated with the low dose of picloram, and the incidence was not significantly different from the controls. However, the test for positive dose-related trend in female rats was significant (pooled controls 0/39, low dose 5/50, high dose 7/49; $p = 0.016$) and the incidences in the high dose group was significant ($p = 0.014$) when compared with that in the pooled-control group.

There was one hepatocellular carcinoma in a low dose male rat and one in a high dose female rat.

There was evidence that picloram affected the livers of rats of both sexes as shown by the incidence of foci of cellular alteration. The incidence in female rats was 1/10 in matched controls, 8/50 in the low dose and 18/49 in the high dose. In male rats, the incidences were 0/10 in the matched controls, 12/49 in the low dose, and 5/49 in the high dose.

No tumors were found in male or female mice or male rats at incidences that could be significantly associated with treatment. It was concluded that picloram was not carcinogenic for B6C3F1 mice or male Osborne-Mendel rats. In female rats, however, the incidence of neoplastic nodules (benign tumors) was associated with treatment with picloram. It was concluded that under the conditions of the bioassay, the findings were suggestive of the ability of picloram to induce benign tumors in the livers of female Osborne-Mendel rats.

Investigators at the NCI have devised a classification scheme for degrees of experimental evidence for the carcinogenicity of chemicals (Griesmer and Cueto 1980). According to this scheme, picloram was listed among chemicals where evidence for carcinogenicity in animals was equivocal at best.

Based on his examination of the histological sections, Reuber (1981) interpreted the results of the NCI Bioassay differently. He concluded that picloram was carcinogenic for all tissues examined in male and female rats given both the low and high doses of picloram, and that mice of both species exposed to the high dose of picloram developed neoplasms of the spleen. Low dose mice were unaffected.

Although adding to the controversy regarding the possible carcinogenicity of picloram, the interpretation by Reuber is included here as an opinion differing from the panel of experts (the former NCI Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens) who were responsible for evaluating the bioassay experiment and interpreting the results. Resolution of the controversy must await the results of the ongoing 2-year chronic study in rats (Dow Chemical Company 1984).

2.5 Mutagenicity

Picloram has been tested in a variety of microbial tests and was generally found to be nonmutagenic. The results of mutagenicity tests of picloram summarized in Appendix E show negative activity in most of the systems tested. Picloram was inactive in the Ames Salmonella typhimurum system, currently the most validated and "standard" bacterial mutagenicity test, (Carere et al. 1976 and Anderson et al. 1972). Negative results were also obtained when picloram was tested in systems employing Escherichia coli bacteriophage (Anderson et al. 1972) or the mold Aspergillus nidulans (Aulicino et al. 1976; Bignami et al. 1977; and Morpugo et al. 1979).

In one of the studies where activity was reported, picloram induced a mutation to streptomycin resistance in a strain of Streptomyces coelicolor previously sensitive to streptomycin (Carere et al. 1976, 1978). These authors were the first to report the use of this novel test to study the mutagenicity of environmental chemicals. The test has not been validated and thus is not ordinarily used in a standard battery of tests for mutagenicity. Similarly, treatment of the yeast Saccharomyces cerevisiae, strain 632/4, with 1 ppm picloram resulted in an increase in the number of revertant mutants which no longer required methionine for growth. However, the increase in mutation frequency was not statistically significantly different from that observed in the untreated controls. Negative results were obtained when either the methionine-dependent strain, 632/1b or the histidine-dependent strain, 814-18b, of S. cerevisiae was similarly treated with picloram (Guerzoni et al. 1976).

In a study to determine possible cytogenetic effects on bone marrow cells in animals, picloram was fed to rats at dosages up to 2,000 mg/kg without any adverse effects (Johnston et al. 1976).

3.0 Environmental Fate

The effectiveness of picloram as a herbicide has been attributed to its inherent potency, its ability to be translocated within the plant, and its relative resistance to degradation at ambient temperatures. Although studies indicate that picloram is not readily metabolized as a primary energy source, the compound is subject to biochemical and physical degradation in the environment in plants, soils, water, and microorganisms (Goring and Hamaker 1971).

3.1 Bioaccumulation

3.1.1 Plants

Picloram is readily absorbed by plant roots and less readily absorbed by foliage. Once absorbed, picloram is readily translocated throughout the plant with a tendency to accumulate in new growth. Although relatively little is known about the metabolism and fate of picloram in plants, most studies indicate that the compound is quite stable and remains largely intact within the plants (National Research Council of Canada 1974; U.S. Department of Agriculture 1973; and Witt and Baumgartner 1979).

3.1.2 Animals

Picloram does not bioaccumulate appreciably in animals and is rapidly excreted, virtually unchanged, primarily in the urine. The metabolism and distribution of picloram in animals are summarized in Appendix F. Table 3-1 indicates bioconcentration factors observed for several organisms exposed to picloram. Studies with radiolabelled picloram (tagged with radioactive carbon atoms) showed that 90 percent of the compound fed in the diet to dogs was excreted within 48 hours in the urine, with small amounts appearing in the feces (Redemann 1963, in National Research Council of Canada 1974). When rats were fed picloram, it appeared unchanged in the urine reaching a peak in two hours. The concentration of picloram in the urine was 100 times the concentration in blood and 10 times that in the stomach. Tissue levels reached a maximum at 2-3 hours, falling rapidly to undetectable levels in 12 hours (Redemann 1964 in National Research Council of Canada 1974). In cows, 97 percent of an ingested dose of picloram was found unchanged in the urine (Fisher et al. 1965). No measurable residues were found in milk samples from dairy cows fed 10-100 ppm picloram in the feed. Milk samples from cows fed 150-1,000 ppm picloram in the feed contained low levels (0.05-0.29 ppm) of residue which declined rapidly and were undetectable 58 hours after withdrawal from the feed.

Nolan et al. (in press) followed the fate of picloram (sodium salt) in human males following ingestion of 0.5 and 5.0 mg/kg doses

Table 3-1

Concentration Factors Observed for Picloram in Some Aquatic and Terrestrial Species

Organism	Medium	Compound	Tissue	Concentration Factors Based On Acid Equivalent of Picloram[a]	Time Scale	References
Daphnia	Water	K-salt[b]	Whole body	1.0	7 weeks	Hardy 1966
Mosquito Fish	Water	Acid	Whole body	0.02	18 days	Youngson and Meikle 1972
Dairy Cattle	Diet	K-salt	Milk	0.0003	2 weeks	Kutschinski 1969
Steers[c]	Diet	Acid	Blood muscle and fat kidney	0.001 0.0005 0.01	2 weeks	Kutschinski and Riley 1969
Sheep	Diet	Acid	Blood	0.001	1 week	McCollister and Leng 1969

[a]Concentration in tissue (acid equivalent) divided by concentration in water or diet (acid equivalent). A factor of one or less means no accumulation greater than that found in the medium of exposure, i.e., diet or water, over the time scale of the exposure. These numbers are not considered absolutes but only indicators since time-dose-dependent studies per se have not generally been carried out.

[b]K-salt = potassium salt.

[c]Upon continuous exposure, residues reached a plateau within three days. The concentration factor, ppm-blood divided by ppm-diet, was independent of time for the remainder of the study and the concentrations in the blood are nearly directly proportional to the concentrations in the feed.

Source: National Research Council of Canada 1974.

and dermal doses of 2.0 mg/kg. In excess of 90 percent of the oral dose was recovered in the urine within 72 hours, most of which (> 75 percent) was excreted within 6 hours. Of the dermally applied picloram, only a small fraction (0.2 percent) was absorbed as determined by urinary output, and this absorption was relatively slow.

3.2 Soil

The fate of picloram in soil is determined by several factors including volatilization, photodecomposition, adsorption and leaching, runoff, and chemical and microbial degradation. Volatilization is not considered to be a major determinant of environmental fate because of the low vapor pressure of picloram.

Picloram is degraded by natural sunlight and ultraviolet light, although the extent of photodecomposition under field conditions has not been quantified. Under laboratory conditions, however, it has been found that 15 percent of picloram applied to soil surfaces was degraded by Texas sunlight after one week (Merkle et al. 1967).

Picloram is generally considered to be a mobile herbicide since its absorption to soil particles is low. Factors which affect adsorption include soil type, pH, rainfall, formulation type and application rate. However, it can be stated that factors which generally result in relatively low adsorption (and, consequently, relatively high mobility) are: low content of organic matter in the soil; low content of hydrated oxides of aluminum and iron in the soil; neutral or high pH (basic); and highly permeable, sandy, light-textured soils (Biggar and Cheung 1973; Farmer and Aochi 1974; Ghassemi et al. 1981; Grover 1971; Hamaker et al. 1963; McCall et al. 1972; National Research Council of Canada 1974; and Norris 1970).

Preliminary studies with various soil types indicate that picloram is usually confined to the upper 1 foot (30 cm) when application rates are low (less than 1 lb/acre [1.12 kg/ha]), but that picloram can readily move to depths greater than 3 feet (approximately 1 meter), even in relatively dry areas, when the application rate is high (3 to 9 lb/acre [3 to 10 kg/ha]) (personal communication cited in National Research Council of Canada 1974).

The persistence of picloram in soils is considered to be moderate to high, since it may exist at phytotoxic levels for a year or more following normal application (Mitchell 1969 and National Research Council of Canada 1974). Picloram persistence in soil is related to both treatment rate and climate. The half-life of the compound has been reported to range from more than four years in arid regions to approximately one month under highly favorable conditions

of moisture, temperature, and organic content of the soil (National Research Council of Canada 1974). Fryer et al. (1979) found picloram residues in a sandy loam soil 1 year after application at 0.04 and 0.22 lb a.i./acre (0.05 and 0.25 kg a.i./ha). At application rates of 1.5 lb a.i./acre (1.68 kg a.i./ha), 0.5 percent of the total amount of picloram applied remained in the soil as residue after 222 weeks.

Since picloram degradation in soil does occur by microbial routes (Hance 1967), degradation increases under conditions which favor microbial growth. Consequently, persistence is generally shorter in soil with high organic content (Bratkowski 1980 and Witt and Baumgartner 1979), high temperatures (Caro et al. 1974), and high soil moisture (Hunter and Stobbe 1972). Studies also indicate that low pH (Youngson et al. 1967), the presence of light-textured sandy soils, and the presence of plant roots also decrease the persistence of picloram (Herr et al. 1966; Hunter and Stobbe 1972; Meikle et al. 1966 and Merkle et al. 1967).

3.3 Water

Because of its mobility, picloram may be transported by surface runoff to nontarget areas including ponds and rivers. However, only a small amount of the total picloram applied is actually removed by runoff. The actual quantity of picloram deposited in lakes has not been reported, but limited studies indicate that rapid mixing and extensive dilution result in negligible residues in streams. It is improbable that aquatic microorganisms will degrade picloram in surface waters to an appreciable extent since microbial degradation rate in soils are fairly low (Youngson et al. 1967). Photodegradation of picloram, which has been demonstrated by some researchers, may be an important mechanism in the dissipation of the compound (National Research Council of Canada 1974; Gear et al. 1982; and Johnsen and Warskow 1980). The major photodegradation pathway in the presence of sunlight or ultraviolet light is dechlorination (Gear et al. 1982). Johnsen and Warskow (1980) found that sunlight decomposed 57 percent of picloram in containers after less than 9 hours of exposure, although in natural soil environments picloram would not be directly exposed to sunlight and thus would not be photodegraded so rapidly.

Levels in farm ponds adjacent to plots treated with picloram at 1 lb/acre (1.1 kg/ha) have reached 1 ppm (Haas et al. 1971 in National Research Council of Canada 1974). However, these levels decreased to less than 10 ppb within 100 days primarily due to dilution and photodegradation of picloram.

Runoff can result after the application of picloram, since the compound (especially in salt formulations) is water soluble. It is generally accepted that there is a potential for high concentrations of picloram in runoff if heavy rainfall occurs soon after application (Gwinn 1975). Maximum concentrations of 400-800 ppb have been detected in surface runoff in instances where heavy rainfall occurred immediately after spraying a 1:1 mixture of triethylamine salts at 1 lb/acre (1.12 kg/ha) on grassland watersheds (Bovey et al. 1967). Runoff accounts for less than 3 percent of the total quantity of picloram applied to soil and the concentration of picloram in runoff generally decreases with time as well as with the time lapse between application and the first rainfall (Trichell et al. 1968 in National Research Council of Canada 1974). Other factors which decrease the concentration of picloram in runoff include decreases in the slope of the terrain, the use of slow release granular formulations rather than liquids, and the distance over which the runoff flows.

In studies of picloram injection into a stream system, Johnsen and Warskow (1980) found that the highest recovery of 38 percent of injected picloram was located 0.25 miles (0.4 km) from the point of injection. At a point 1 mile (1.6 km) downstream of the injection site, less than 5 percent of the injection concentration was found in water samples.

Aerial application of a mixture of picloram at 2.5 lb active equivalent (a.e.) per acre (2.8 kg/ha) and 2,4-D at 5 lb a.e./acre (5.6 kg/ha) onto 279 acres (113 ha) of a pinyon-juniper watershed resulted in detectable levels of picloram in runoff for 30.5 months (Johnsen 1980). The highest concentration of picloram detected was 320 ppb in the initial runoff event following treatment. Of the total picloram applied, 1.1 percent ultimately left the area in runoff water.

Neary et al. (1979) monitored streamflow from a 10-acre (4-hectare) watershed for picloram residues following application of Tordon 10K® pellets at a rate of 4.5 lb a.i./acre (5 kg/ha) for site preparation. Picloram was applied in mid-May and two pulses of residues were detected in watershed outflow; a 3 ppb pulse in early June following precipitation and a maximum 8 ppb pulse in early July (no precipitation). No further residues were detected (reported through October) at detection limits of 1 ppb although there were relatively heavy rains in early August.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to picloram during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved picloram. Calculations of exposure to picloram in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to picloram are brief, and that any area sprayed in a given year may not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Picloram Application

Available application data for picloram use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to picloram differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of various picloram formulations in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Because of the relative persistence of picloram in soil and water environments, forest workers, visitors, and wildlife may be exposed to residual picloram in the environment for a longer period of time than they would to less persistent herbicides. On the other hand, however, the relatively low mobility of picloram, coupled with its lack of bioaccumulation, tends to reduce the availability of residual picloram in the environment and hence tends to reduce the potential for incidental exposure of humans and wildlife to only low levels of picloram.

4.2 Exposure

Estimates of potential exposure to picloram are based on aerial as well as ground application. The maximum aerial application rate

Table 4-1

USDA Forest Service Application Data for Picloram (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Beads (15)	Hand Broadcast	2.4	36	4.8	7.8	6.9	12.5	Range (3) Noxious Weeds (12)
K (4)	Aerial	5.3	22	1.4	5.8	2.9	9.5	Timber (4)
2K (29)	Hand Broadcast	3.3	96	4.5	10.8	6.6	10.1	Range (20) Noxious Weeds (9)
10K (27)	Hand Broadcast	5.9	160	5.5	8.2	6.6	11.2	Wildlife (13), Range (8) Timber (5) Fuels Management (1)
22K (42)	Ground Foliar	3.3	139	5.9	10.3	7	12.3	Range (18) Noxious Weeds (19) Right-of-Way (5)
22K (1)	Aerial	2		4	1	4	1	Noxious Weeds (1)
101 (3)	Aerial	4.3	13	3	6.7	4.7	9.7	Right-of-Way (2) Timber (1)
101 (11)	Injection, Cut Stump	16.3	180	4.8	29.5	6.6	40.4	Timber (10) Recreation (1)
101 (8)	Ground Foliar	3.2	26	5	8.6	7.5	8.5	Timber (3), Range (3) Noxious Weeds (2)
101R or RTU (19)	Injection, Cut Stump	13.4	255	6.4	33.9	6.8	49.8	Timber (12) Wildlife (7)
212 (12)	Ground Foliar	2.5	30	5.3	10.0	6.7	9.4	Range (11) Noxious Weeds (1)

[a]Grand Total = 957

Source: Gross 1983

for picloram (see Table 1-1) is 1.9 lb/acre, while the compound is applied from the ground (ground foliar) at rates as high as 10 lb a.e./acre. In both aerial application and ground foliar application, picloram is applied as a liquid. The calculations in Table 4-2 that are based on aerial application (e.g., spray observer dose, wildlife dermal exposures, and water concentrations) assume an application rate of 1.9 lb a.e./acre. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose and wildlife oral exposures) assume an application rate of 10 lb a.e./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of picloram and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of picloram to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for picloram are based on exposures on a per pound per acre application rate multiplied by 1.9 lb/acre for observers and 10 lb/acre for backpack sprayers. In the case of the aerial spray observer, exposure estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be 1 percent based upon the data of Nolan et al. (in press) indicating 0.2 percent of dermally applied picloram to be absorbed in humans. At 1.9 lb a.i./acre application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.0076 mg/kg (0.004 mg/kg at 1 lb/acre application rate since 1 percent dermal absorption is assumed for picloram)

For ground applications at 10 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.40 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Picloram

	Exposure Route[a]		
Occupational Dose	Dermal	Inhalation	Oral
Mixer/Loader Observer Backpack Sprayer	0.1 mg/kg up to 0.0076 mg/kg 0.40 mg/kg		[b]
Environmental Exposure			
Fish	0.019 ppm (Immersion)		[c]
Rabbits Deer	0.756 mg/kg 0.192 mg/kg	Negligible Negligible	10.50 mg/kg 14.40 mg/kg
Man	--[d]	--[d]	--[d]
(Fish) (Rabbits) (Deer) (Water) (Berries) (Mushrooms)	NA[e] NA NA NA NA NA	NA NA NA NA NA NA	0.00038 mg/kg (2 days) 0.042 mg/kg (2 days) 0.058 mg/kg (2 days) 0.00076 mg/kg (1 day) 0.0019 mg/kg (1 day) 0.00095 mg/kg (1 day)
Toxicity Summary	LD_{50} > 2,000 mg/kg (Tordon 22K® in rabbits)	Saturated atmosphere nontoxic (Tordon 22K® in rabbits)	Acute LD_{50}'s from 2,000 to 8,200 mg/kg (rabbit, mouse, guinea pig, rat). Chronic NOEL of 150 mg/kg (rat and dog).
	LC_{50} >10 ppm for fish		

[a]Assumes aerial application at 1.9 lb a.e./acre and ground foliar application at 10 lb a.e./acre. See text for further explanation.

[b]Occupational doses include all routes of exposure and are on a daily basis.

[c]Fish exposure results from immersion in water containing picloram.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by picloram application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less by orders of magnitude than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 1.9 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 0.7657 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.1919 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation of picloram vapor would be negligible since the vapor pressure of picloram is low. Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds

adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of picloram in water, calculated as:

Water concentration: 0.019 ppm (0.01 ppm at 1.0 lb/acre application rate)

This concentration of picloram represents the estimated maximum concentration immediately after spraying. If heavy rainfall occurred immediately after spraying, higher transient concentrations could occur in streams and ponds. However, such high concentrations result from high application rates and from precipitation soon after application, and would be transient since they are rapidly diluted by resident waters. Monitoring data reviewed in Section 3.3 confirm the low levels of picloram in waterbodies following application of picloram to watersheds.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of picloram by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 10 lbs a.e./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 14.40 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 10.50 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of picloram in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.019 ppm
- bioconcentration factor: 1.0 (measured bioconcentration < 1)

Fish tissue concentration: 0.019 ppm (0.019 mg/kg)

Deer and rabbit tissue concentrations include picloram accumulated from both oral and dermal exposure. Tissue concentrations estimates for deer and rabbit assume that dermally applied picloram is absorbed at a rate of 1 percent. It is also assumed that oral exposure of

deer and rabbit to picloram lasts only for 2 days due to the relatively short environmental persistence of picloram and that only 10 percent of the ingested material is assimilated. Deer tissue concentrations of picloram are derived as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 0.1919 mg/kg (dermal exposure) x 0.01 = 0.001919 mg/kg

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 14.40 mg/kg/day (oral exposure) x 0.1 x 2 days = 2.880 mg/kg

Total Deer Tissue concentration = 0.001919 mg/kg + 2.880 mg/kg = 2.88192 mg/kg (2.882 ppm)

Rabbit tissue concentrations of picloram are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 1 percent absorption of a single exposure, or 0.7657 mg/kg (dermal exposure) x 0.01 = 0.007657 mg/kg

Oral Exposure contribution assumes 10 percent absorption of two-days feeding, or 10.50 mg/kg/day (oral exposure) x 0.1 x 2 days = 2.100 mg/kg

Total Rabbit Tissue Concentration = 0.007657 mg/kg + 2.100 mg/kg = 2.10766 mg/kg (2.108 ppm)

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to picloram from eating meat are derived:

Fish meat: 0.019 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.00038 mg/kg

Deer meat: 2.882 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0576 mg/kg

Rabbit meat: 2.108 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0422 mg/kg

Water and plant consumption by man would result in the following oral exposures to picloram based on assumptions stated in the General Introduction to the Herbicide Background Statements at an application rate of 1.9 lb a.i./acre (aerial):

Water: 0.00076 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries: 0.0019 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms: 0.00095 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of picloram to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rabbit inhalation results, rabbit and rodent acute oral LD_{50}'s, rat and dog chronic oral NOEL's, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for USDA Forest Service applications. Based upon chronic feeding studies, NOEL's of 150 mg/kg are reported in rats and dogs. Assuming a 100-fold margin of safety for extrapolation to man (a factor of 10 for inter-species variability and a factor of 10 for human intra-species variability), one can assume that 150 mg/kg divided by 100, or a 1.5 mg/kg oral dose in man will not result in adverse effects. The estimated human exposures from ingestion of water, game, and plant material are well below 1.5 mg/kg per day.

Appendix A

Summary of Picloram Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Picloram in 60% Sucrose Syrup	Honeybees (Apis mellifera; Insecta)	Ingestion and surface absorption	14 days	NOEL was 1,000 ppm by weight	Highest dose tested was 1,000 ppm by weight, sprayed on cage.	Morton and Moffett 1972
Picloram in 60% Sucrose syrup	Honeybees (Apis mellifera; Insecta)	Ingestion	60 days	No reduction in half-life was observed at the highest dose of 1,000 ppm	The half-life was defined as the number of days required for 50% of the bees to die.	Morton et al. 1972
Tordon 212® and 22K® in water	Honeybees (Apis mellifera; Insecta)	Ingestion and surface absorption	14 days	Mortality was not changed after spraying at a rate of 4 lb a.i./acre	Bees (and their cages) were sprayed directly with a picloram mixture and observed for 14 days.	Moffett et al. 1972
Picloram	Stonefly nymphs (Pteronarcys californica; Insecta)	Surface absorption	24 hours	LC_{50} was 120 ppm	--[a]	Sanders and Cope 1968
Picloram	Amphipod (Gammarus lacustris; Crustacea)	Surface absorption	24 hours	LC_{50} was 50 ppm	--	Sanders 1969
Picloram	Amphipid (Gammarus lacustris; Crustacea)	Surface absorption	48 hours	LC_{50} was 48 ppm	--	U.S. Department of the Interior 1968 in Pimentel 1971
Picloram	Copepod (Daphnia sp.; Crustacea)	Surface absorption	24 hours	95 percent mortality at 530 ppm concentration, NOEL at 380 ppm	--	Lynn 1965
Picloram	Copepod (Daphnia sp.; Crustacea	Surface absorption	10 weeks	NOEL at 1 ppm	No observed effect on growth and reproduction.	Hardy 1966

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Picloram	Brown Shrimp (Crustacea)	Surface absorption	48 hours	NOEL at 1 ppm	--	U.S. Department of the Interior 1966
Picloram	Snails (Mollusca)	Surface absorption	--	100 percent mortality at 530 ppm concentration NOEL at 380 ppm	--	Lynn 1965
Picloram	Eastern Oyster (Mollusca)	Surface absorption	48 hours	NOEL at 1 ppm	No observed effect on shell growth.	Butler 1965
Picloram	Bacterium (Thiobacillus thiooxidans)	Surface absorption	--	Growth inhibited at 1,000 ppm but not at 100 ppm	Rates of carbon dioxide evolution, nitrification and urea hydrolysis were unaffected.	Goring et al. 1967
Picloram	Fungus (Aspergillus niger)	Surface absorption	--	Growth was not reduced at concentrations up to 50 ppm.	Picloram tended to accumulate in mycelia.	Arnold et al. 1966
Picloram	Fungi (Trichoderma virdie, Fusarium oxysporum, Helminthosporium victoriae, Penicillium lanosum, and Aspergillus flaves	Surface absorption	--	All showed growth in cultures with 1 to 1,000 ppm concentration.	--	Hameed and Foy 1974
Picloram	Fungi (Penicillium sp.; Aspergillus sp.; and Trichoderma sp.)	Surface absorption	--	Concentrations of 1 and 10 ppm inhibited populations of Penicillium stimulated Aspergillus and Trichoderma	--	Tu and Bollen 1969

[a]Data are not available or not applicable.

Appendix B

Summary of Picloram Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Picloram	Goldfish	Static bioassay	24 to 96 hours	LC_{50} was 14 to 36 ppm acid equivalent	75°F water temperature	U.S. Department of the Interior 1964 in Kenaga 1969
Picloram	Largemouth Bass	Static bioassay	24 to 48 hours	LC_{50} was 13.1 to 19.7 ppm acid equivalent	75°F water temperature	U.S. Department of the Interior 1964 in Kenaga, 1969
Picloram	Rainbow Trout	Static bioassay	24 to 96 hours	LC_{50} was 24 to 34 ppm acid equivalent	55°F water temperature	U.S. Department of the Interior 1965 in Kenaga 1969
Picloram	Bluegill	Static bioassay	24 to 96 hours	LC_{50} was 21 to 26.5 ppm acid	63°F water temperature	Bond et al. 1967 in Kenaga 1969
Picloram	Coho Salmon	Static bioassay	24 to 96 hours	LC_{50} was 21 to 29 ppm acid equivlent	63°F when fish forced to swim against current an increased mortality was observed LC_{100} was 29 ppm.	Bond et al. 1967 in Kenaga 1969
Picloram	Mosquito Fish	Static bioassay	24 to 96 hours	TL_{50} was 133 to 120 ppm	70 to 72°F water temperature	Johnson 1978
Technical Grade (90% a.i.)	Cutthroat Trout	Static bioassay	96 hours	96 hours LC_{50} was 5.0 ppm	pH: 7.2 HD: 30-40 10°C	Woodward 1976
Technical Grade (90% a.i.)	Lake Trout	Static bioassay	96 hours	96 hours LC_{50} was 4.25 ppm	pH 7.2 HD: 30-40 10°C	Woodward 1976

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Technical Grade (90% a.i.)	Cutthroat Trout	Flow through bioassay	22 days	NOEL to fry ranged from 290 ppb to 48 ppb	pH: 7.4 Alkalinity: 206 ppb 9.5°C	
					Author recommends that stream residues not exceed 290 ppb after first major rainfall following application.	Woodward 1979
Picloram as potassium salt, Technical	Fathead Minnow	Static bioassay	72 hours	LC_{100} < 259 ppm; NOEL observed at 86 ppm	50°F water temperature	
	Brook Trout	Static bioassay	72 hours	LC_{100} < 259 ppm; NOEL observed at 130 ppm	50°F water temperature	Winston 1963 in Kenaga 1969
	Brown Trout	Static bioassay	72 hours	LC_{100} < 259 ppm; NOEL observed at 130 ppm	50°F water temperature	
Picloram as potassium salt, Technical	Pugnose Minnow	Static bioassay	72 hours	LC_{100} < 173 ppm; NOEL observed at 86 ppm	50°F water temperature	Winston 1963 in Kenaga 1969
	Guppy	Static bioassay	6 months	NOEL observed at 1 ppm	75 to 85°F water temperature	Hardy 1966 in Kenaga 1969
	Goldfish	Static bioassay	24 hours	LC_{100} > 35 ppm	70°F water temperature	Hardy 1962 in Kenaga 1969
	Goldfish	Static bioassay	10 weeks	NOEL observed at 1 ppm	75 to 85°F water temperature	Hardy 1966 in Kenaga 1969
	Rainbow Trout	Static bioassay	24 to 96 hours	LC_{50} was 13 to 27 ppm	60°F water temperature	Alexander and Bachelder 1965 in Kenaga 1969

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Picloram as potassium salt, Technical	Bluegill	Static bioassay	24 to 96 hours	LC_{50} was 24 to 69 ppm	80°F water temperature	Alexander and Bachelder 1965 in Kenega 1969
	Channel Catfish	Static bioassay	24 to 96 hours	LC_{50} was 14 to 41 ppm	80°F water temperature	
S-1665 (31.4% a.i. TIIP salt)	Fathead Minnow	Static bioassay	72 hours	$LC_{100} < 157$ ppm; NOEL observed at 63 ppm	50°F water temperature	Winston 1963 in Kenaga 1969
	Brook Trout	Static bioassay	72 hours	$LC_{100} < 157$ ppm; NOEL observed at 94 ppm	50°F water temperature	
	Brown Trout	Static bioassay	72 hours	$LC_{100} < 157$ ppm; NOEL observed at 63 ppm	50°F water temperature	
	Pugnose Minnow	Static bioassay	72 hours	$LC_{100} < 94.2$ ppm; NOEL observed at 47 ppm	50°F water temperature	Winston 1962 in Kenaga 1969
Technical Triisopropanolamine salt	Rainbow Trout	Static bioassay	24 to 96 hours	LC_{50} was 210 to 279 ppm	60°F water temperature	Duddles 1968a in Kenega 1969
Triethylamine salt	Rainbow Trout	Static bioassay	24 to 96 hours	LC_{50} was 29 to 43 ppm	60°F water temperature	
	Channel Catfish	Static bioassy	24 to 96 hours	LC_{50} was 53 to 71 ppm	80°F water temperature	
	Goldfish	Static bioassay	24 to 96 hours	LC_{50} was 44 to 91 ppm	80°F water temperature	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Tordon 22K®	Fathead Minnow	Static bioassay	24 to 96 hours	LC_{50} was 29 to 52 ppm; NOEL observed at 22 ppm (96 hours)	50°F water temperature	Lynn 1965 and Winston 1963 in Kenaga 1969
	Green Sunfish	Static bioassay	24 to 96 hours	LC_{50} was 91 ppm; NOEL observed at 39 ppm (96 hours)	50°F water temperature	
	Black Bullhead	Static bioassay	24 to 96 hours	LC_{50} was 91 ppm; NOEL observed at 69 ppm (96 hours)	50°F water temperature	
	Brook Trout	Static bioassay	24 to 96 hours	LC_{50} was 91 ppm; NOEL observed at 69 ppm (96 hours)	50°F water temperature	
Tordon 22K®	Flagfish	Flow through bioassay	10 days	10 day LC_{50} was 12.3 ppm	pH: 8.0-8.3 HD: 350-375 Alkalinity: 200-255 25°C	Fogels and Sprague 1977
Tordon 22K®	Rainbow Trout	Flow through bioassay	10 days	10 day LC_{50} was 22.2 ppm	25°C 15°C	
Tordon 22K®	Zebrafish	Flow through bioassay	10 days	Threshold LC_{50}[a] was 35.5 ppm	15°C	
Tordon 22K®	Coho Salmon	Static bioassay	--[b]	24 hours LC_{50} was 17.5 ppm	10°C	Lorz et al. 1979
Tordon 22K®	Coho Salmon	Static bioassay	24 hours	LC_{50} was 17.5 ppm	10°C	Spehar et al. 1981

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Tordon 22K®	Bluegill	--	24 to 96 hours	LC_{50} was 5.4 to 8.2 ppm	65°F water temperature	U.S. Department of the Interior 1966 in Kenaga 1969
Tordon 22K®	Brown Trout	--	24 to 96 hours	LC_{50} was 52 ppm; NOEL observed at 22 ppm (96 hours)	50°F water temperature	Lynn 1965 and Winston 1962 in Kenaga 1969
	Rainbow Trout	--	24 to 96 hours	LC_{50} was 50 to 58 ppm; NOEL observed at 22 ppm (96 hours)	50°F water temperature	
Tordon 22K®	Lake Emerald Shiner	--	4 to 96 hours	LC_{50} was 30 to 65 ppm	68-78°F water temperature	Swabey 1963 in Kenaga 1969
Tordon 22K®	Bluegill	--	24 to 48 hours	LC_{50} was 43 ppm	77°F water temperature	Hughes and Davis 1966 in Kenaga 1969
M 2437 potassium salt with increased Surfactant	Green Sunfish	--	24 to 96 hours	LC_{50} was 26 to 29 ppm	50°F water temperature	Winston 1962 in Kenaga 1969
	Pugnose Minnow		24 to 96 hours	LC_{50} was 16 ppm		
Tordon 101®	Coho Salmon	Static bioassay	24 hours	LC_{50} was 20 ppm	pH: 7.4-7.6 HD: 102 10°C	Lorz et al. 1979
Tordon 101®	Coho Salmon	Static bioassay	24 hours	LC_{50} was 17.5 ppm	pH: 7.4-7.6 HD: 102 ppb 10°C	Spehar et al. 1981

[a]Threshold LD_{50} was judged to have been attained when 48 hours elapsed with no mortality in a test tank. If this did not happen in 10 days, a bioassay was terminated on the basis that there was not definite threshold.
[b]Data not available or not applicable.

Appendix C

Summary of Picloram Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Technical Grade (90.5%)	Mallard Duck (males)	Ingestion	Single dose	14 days LD_{50} was >2,000 mg/kg	Birds were 3-4 months old	
	Pheasants (males)	Ingestion	Single dose	14 day LD_{50} was >2,000 mg/kg	Birds were 3-4 months old	
Tordon 22K®	Mallards Duck	Ingestion	Single dose	14 day LD_{50} was >2,000 mg/kg	Birds were 7 months old	Tucker and Crabtree 1970
Unspecified	Bobwhite Quail	Ingestion	5 days	LC_{50} >5,000 ppm	All test birds were incubator-hatched progeny of breeding colonies	
	Japanese Quail	Ingestion	5 days	LC_{50} > 5,000 ppm		
	Pheasant	Ingestion	5 days	LC_{50} > 5,000 ppm	All colonies were randomly outbred so that findings could be more readily related to wild populations	Heath et al. 1972
	Mallard Duck	Ingestion	5 days	LC_{50} >5,000 ppm		
Technical	Japanese Quail	Fed diets containing 100, 500, 1,000 mg/kg (feed) of picloram for three generations	—[a]	No adverse effect on food consumption, egg production, hatchability, fertility, survival or body weight	Initially F_0 birds were placed on picloram diet when 2 days old, for 20 weeks	Kenaga 1969

Appendix C (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Technical	Japanese Quail	Birds feed diets containing 100 or 1,000 mg picloram/kg feed for 2 weeks	--	At 100 mg/kg feed, no effect on feathering, plumage, egg production, fertility or body weight. At 1,000 mg/kg a decrease in egg fertility and hatchability occurred in the first week of treatment	Adult birds treated.	Kenaga 1969
2,4-D: Picloram (4:1)	Single Comb White Leghorn Chicken	Absorption through egg incubation.	One spray 24 hours before	NOEL was observed at 10 times normal application rate (28 kg/ha = 24.9 lb/acre)	Eggs were examined for adverse effects during incubation (a decrease in percent of viable embryos) and early chick performance (weight gain)	Somers, Moran, Jr. and Reinhart 1973
2,4-D: Picloram (4:1)	Single Comb White Leghorn Chicken	Absorption through egg	One spray 24 hours before incubation	NOEL was observed at application rate of 2.8 kg/ha (2.49 lb/acre)	Treatments were conducted to determine adverse effects on hatching success, incidence of malformed embryos, and chick.	Somers, Moran, Jr. Reinhart and Stephenson 1974
2,4-D: Picloram (4:1)	Ring-neck Pheasant	Absorption through egg	One spray 24 hours before incubation	NOEL was observed at the application rate of 2.8 kg/ha (2.49 lb/acre)	Treatments were conducted to determine adverse effects on hatching success, incidence of malformed embryos, and chick mortality.	Somers, Moran, Jr. and Reinhart 1974

Appendix C (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
2,4-D: Picloram (4:1)	Single Comb White Leghorn Chicken	Absorption through egg	One spray 24 hours before incubation.	NOEL was observed at 10 times normal application rate (28 kg/ha = 24.9 lb/acre)	Eggs and birds were examined for adverse effects during incubation (a decrease in percent of viable embryos) and early chick performance (weight gain).	Somers, Moran, Jr. and Reinhart 1974
Tordon 22K®	Single Comb White Leghorn Chicken	Absorption through egg	3 days	NOEL was observed at 10 times normal application rate (11.2 kg/ha = 9.98 lb/acre) 0, 9, and 18.	An aqueous solution was sprayed on eggs at a concentration 10 times normal (11.2 kg/ha = 9.98 lb/acre) on days	Somers et al. 1978a
Tordon 22K®	Single Comb White Leghorn Chicken	Absorption through egg	3 days	NOEL was observed at 10 times normal application rate (11.2 kg/ha = 9.98 lb/acre) over the period of one generation and into the early development stage of the second generation.	A solution of picloram at a concentration 10 times normal (11.2 kg/ha = 9.98 lb/acre) was sprayed on first generation eggs on days 0, 4, and 18 and on second generation eggs on day 0. Hens and cockerels from the sprayed fertile eggs were evaluated for reproductive effects (egg production, egg weight, shell porosity, shell strength, sperm counts, and weight of testis, reproductive success, embryonic mortality, malformations, and weight gain and deaths of chicks).	Somers, Moran, Jr. and Reinhart 1978b

[a]Data not available or not applicable.

Appendix D

Summary of Picloram Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Technical Grade	Rat	Ingestion of a single dose	LD_{50} = 8,200 mg/kg	--[a]	Lynn 1965
	Mouse		LD_{50} = 2,000 to 4,000 mg/kg		
	Guinea pig		LD_{50} = approx. 3,000 mg/kg		
	Rabbit		LD_{50} = approx. 2,000 mg/kg		
	Chicken		LD_{50} = approx. 6,000 mg/kg		
	Sheep		NOEL = 650 mg/kg		
	Calves		NOEL = 488 mg/kg		
Tordon 22K® (25% potassium salt)	Rat	Ingestion of a single dose	LD_{50} = 10,300 mg/kg	--	Olson 1963 in National Research Council of Canada 1974; Lynn 1965; and Jackson 1965
	Sheep		NOEL = 4,650		
	Calves		NOEL = 3,480		
Isooctyl ester	Rat	Ingestion of a single dose	LD_{50} = 3,250 mg/kg for males LD_{50} = 2,830 mg/kg for females	--	Dow Chemical Company 1984
			Acute Dermal Toxicity		
Technical Grade	Rabbit	Applied to shaved skin at dosages up to 4g/kg body weight for 24 hours	LD_{50} > 4g/kg. No mortality or toxic signs; skin showed slight edema and brown discoloration.	--	Lynn 1965
Tordon 22K®	Rabbit	Applied to shaved skin and dosages up to 2 g/kg weight for 24 hours	LD_{50} > 2 g/kg. No observable effects noted.		
Isooctyl ester	Rabbit	--	LD_{50} < 2 g/kg		Dow Chemical Company 1984

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Inhalation Toxicity		
Tordon 22K®	Albino Rat	Inhalation of a saturated atmosphere for 7 hours.	LC_{50} not reached. No adverse effects observed during exposure or for 2 weeks following exposure.	--	Lynn 1965
			Eye Irritation		
Technical Grade	Albino Rabbit	Undiluted and 10% slurry of test substance place into the conjunctival sac	Slight to moderate conjunctival redness or conjunctivitis which disappeared in 1 to 2 days	--	Lynn 1965
Tordon 22K®	Albino Rabbit	Undiluted test substance placed into conjunctival sac	Conjunctival redness and very slight corneal cloudiness. Subsided in 24 to 48 hours.		
Isooctyl ester	Albino Rabbit	Undiluted test substance placed into conjunctival sac	Nonirritating	--	Dow Chemical Company 1984

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Toxicity		
Technical	B6C3F1 Mouse	1,000, 1,400, or 2,000 mg/kg for 13 weeks	No clinical or hematologic changes. Decreased body weights of females at 2,000 mg/kg. Decreased alkaline phosphatase in all groups. Increased liver weights in females at all doses and males at 1,000 mg/kg/day or above. Dose-related morphologic changes in hepatocytes of females at all doses and males at 1,400 mg/kg/day or above. Liver changes in males at 1,000 mg/kg/day. NOEL not determined.	--	Dow Chemical Company 1984
Technical	Mouse	30, 100, 650, 1,000, or 3,000 mg/kg/day for 32 days	Effects on livers and gastric mucosa at 3,000 mg/kg/day. NOEL was 1,000 mg/kg/day.	--	Dow Chemical Company 1984
Technical	Rat	1,000, 3,000 and 10,000 ppm equivalent to 75, 75, 225, 750 mg/kg/day fpr 90 days in diet	No toxic effects at 1,000 ppm; moderate changes in liver and kidneys and slight decrease in body weight at 3,000 and 10,000 ppm.	Mortality, body weight changes, food consumption, hematology, clinical chemistry, organ to body weight changes were evaluated	Lynn 1965
Technical	Rat	15, 50, 150, 300, and 500 mg/kg/day for 13 weeks	No toxic effects except increased liver weights and minor liver changes; LEL = 150 mg/kg; NOEL = 50 mg/kg.	--	Dow Chemical Company 1984

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Toxicity Continued		
Technical	Dog (beagle)	7, 35, or 175 mg/kg/day for 13 weeks	Decreased body weights, body weight gain, food consumption, and alanine transaminase and increased alkaline phosphatase, and liver weights at 175 mg/kg. NOEL = 7 mg/kg/day.	--	Dow Chemical Company 1984
Technical	Sheep	18 mg/kg body body weight per day in diet for 33 days	No toxic signs or adverse effects on growth	Yearling animals	Jackson 1965
Technical	Beef Cattle	7.2 or 15.4 mg/kg body weight per day in diet for 31 days	No toxic effects	Calves	Lynn 1965
Technical	Swine	22 mg picloram/kg feed for unspecified time	Stimulated growth and improved feed efficiency	--	McCollister and Leng 1969
Tiipa[b] salt	Rat	Up to 0.3% in the diet (equivalent to 0.16% of the acid) for 90 days	No toxic effects. Gross and histopathology negative.	--	Lynn 1965; McCollister and Leng 1969
Tordon 22K®	Sheep	72 mg/kg body weight/day acid equivalent for 30 days	No toxic signs or adverse effects on growth	--	Jackson 1965
Tordon 22K®	Sheep	0.42 mg /kg/day (100 mg acid equivalent/kg/day) for 30 days	No observable effects	Yearling animals	Lynn 1965

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Toxicity (Concluded)		
Potassium salt	Sheep	100 mg/kg body weight in the diet	No toxic effects after 10 doses	--	
		250 mg/kg body weight	One of 3 animals dead after 9 doses; 2 animals of 10 survived 10 doses with a loss in weight.	--	Palmer and Radeleff 1969
	Beef Cattle	100, 250 mg/kg body weight for 10 doses. 500 mg/kg body weight for 8 doses.	No toxic effects; NOEL was 250 mg/kg body weight. Two of 2 animals died. When fed 500 mg/kg body weight.	--	Palmer and Radeleff 1969
Potassium salt (22.8 a.e.)	Steers	2 to 1,600 mg/kg and equivalent in feed, for up to 8 weeks	No adverse effects	Calves weighting 400 to 600 pounds.	Kutschinski and Riley 1969
			Subchronic Dermal Irritation		
Technical	Albino Rabbit	Undiluted material applied 9 times to the shaved skin over a period of 11 days	Slight exfoliation and hyperemia in exposed area	--	Lynn 1965
Tordon 22K® (24.9% potassium salt)		Saturated cotton pad applied to skin 10 times over a period of 14 days	Slight hyperemia and edema of treated areas in all animals; slight eschar in one animal.	--	Lynn 1965

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Dermal Irritation Concluded)		
Tordon 22K®		Repeat and challeng applications of a 10% solution	No skin irritation or skin sensitization	Only summary statement given. No details of protocol were given.	Lynn 1965
Isooctyl ester	Albino Rabbit	10 applications to intact or abraded skin	Slight swelling, redness, and superficial necrosis which appeared sooner on abraded skin	Only summary statement given. No details of protocol were given.	Dow Chemical Company 1984
6% Tordon +22% 2,4-D acid	Human	Unspecified	No dermal irritation or sensitization	--	Dow Chemical Company 1984
10.2% picloram tiipa[a] salt + 39.6% 2,4-D tiipa[a] salt	Human	Unspecified	No dermal irritation or sensitization	--	Dow Chemical Company 1984
10% picloram salt	Human	Unspecified	No dermal irritation or sensitization	--	Dow Chemical Company 1984
			Chronic Toxicity		
Technical	Dog (beagle)	15, 50, 50 mg/kg body weight/day in diet for 2 years	LD_{50} 150 mg/kg/day; no detrimental effects observed.	Endpoints examined included body weight, food consumption, behavior, mortality, hematology, clinical chemistry, urinalysis	Lynn 1965 and McCollister and Leng 1965

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity (Concluded)		
Technical	Rat	15, 50, 150 mg/kg body weight/day in diet for 2 years	LD_{50} 150 mg/kg/day. No toxic effects observed.	Studies not validated because raw data destroyed	Dow Chemical Company 1984
			Reproductive and Teratogenicity		
Technical	Rat	0.03 to 0.3% (equivalent to 15 to 150 mg/kg body weight day) in diet for three generations. Initially for 28 days prior to first mating of F_0 generation	No significant differences between treatment and control groups regarding gestation, viability, lactation, weanling weights and resorption rates	--	McCollister and Leng 1969 and Dow Chemical Company 1984
Technical	Mice	0.01 percent (equivalent 15 mg/kg/body weight) picloram in diet for 4 days before and 14 days after mating	No adverse effect on fertility or litter size	--	McCollister and Leng 1969
Picloram, production grade	Rat	Oral treatment with 500, 750, or 1,000 mg/kg body weight/day on days 6 to 15 of gestation	Toxic signs observed at 750 and 1,000 mg/kg/day between 7 and 17 days. No adverse effects on gestation period, number of implanations, corpora lutea and resorption per dam, litter size, fetal weight, pup weight, sex ratio, viability and lactation at 500 or 750 mg/kg/day. Decrease in viability index at 1,000 mg/kg/day, some minor skeletal abnormalities seen in groups treated with 750 or 1,000 mg/kg/day related to maternal toxicity.	--	Thompson et al. 1972 and Dow Chemical Company 1984

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity		
Picloram, Technical Grade	Osborne-Mendel Rat	Groups of 50 rats of each sex fed picloram in diet for 80 weeks as time-weighted doses of 7,437 or 14,875 ppm. Observed for additional 33 weeks. All survivors killed at 113 weeks	Not carciongenic in male rats. Hepatic neoplastic nodules (benign tumor) in female rats	High, incidence of follicular hyperplasia and C-cell adenomas of the thyroid in both sexes. Adenoma incidence not statistically significant. Increased incidences of hepatic neoplastic nodules, considered to be a benign tumor, in male and female animals. Positive dose related trend in females suggestive of the ability of the compound to induce benign liver tumors in female Osborne-Mendel rats.	National Cancer Institute 1978
Picloram, Technical Grade	B6C3F1 Mouse	Groups of 50 mice of each sex fed picloram in diet for 80 weeks at time-weighted doses of 2,531 or 5,062 ppm	Noncarcinogenic in male and female mice	No tumors found in male or female mice at incidences significantly associated with treatment	National Cancer Institute 1978
Picloram, Technical Grade	Osborne-Mendel Rat	Groups of 50 rats of each sex fed picloram in diet for 80 weeks at time-weighted doses of 7,437 or 14,875 ppm	Neoplasms at all sites, including malignant neoplasms, were increased in both low- and high-dose treated male and female rats. The malignant neoplasms were both carcinomas and sarcomas.	Author's interpretation of NCI Bioassay summarized in NCI Carcinogenesis Tech. Rept. Series No. 23	Reuber 1981

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Carcinogenicity (Concluded)		
Picloram, Technical Grade	B6C3F1 Mouse	Groups of 50 mice of each sex fed picloram in diet for 80 weeks at time-weighted doses of 2,531 or 5,062 ppm	Carcinogenic for spleen of males and females	Author's interpretation of NCI Bioassay summarized in NCI Carcinogenesis Rept. Series No. 23	Reuber 1981
Picloram, Technical Grade	Albino Rat	Animals fed picloram in diet at daily levels of 15, 50, and 150 mg/kg/day for 2 years	Noncarcinogenic in male or female rats	Information given a summary paragraph. No data presented. Cannot be validated because raw data destroyed.	Lynn 1965; McCollister and Leng 1969; and Dow Chemical Company 1984
Picloram, Technical Grade	Dog (beagle)	Animals fed picloram at daily doses of 15, 50, and 150 mg/kg for 2 years	No gross or histopathological changes attributed to treatment	Summary statement. No data presented. Cannot be validated because raw data destroyed.	Lynn 1965; McCollister and Leng 1969; and Dow Chemical Company 1984
Picloram Technical	Osborne-Mendel Rat; B6C3F1 Mouse	See NCI Carcinogenesis Rept. Series No. 23	Equivocal evidence for carcinogenicity	Interpretation of results from NCI Bioassay summarized in NCI Carcinogenesis Tech. Rept. Series No. 23	Griesemer and Cueto 1980

[a]Triisopropanolamine
[b]Data not available or not applicable.

Appendix E

Summary of Mutagenicity Studies with Picloram

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Picloram	Salmonella typhimurium, 8 strains, unspecified	Not given	Negative	No metabolic activation system was used, therefore only direct acting mutagens could be detected.	Anderson et al. 1972
	T_4 bacteriophage of Escherichia coli B	Not given	Negative	--[a]	
	Mutants of T_4 bacteriophage AP72	6,000 µg/plate	Negative	--	Anderson et al. 1972
	Mutants of T_4 bacteriophage, N17	500 µg/plate	Negative	--	
Picloram, Tordon®	Aspergillus nidulans	Not given	Negative	Doses used, negative and positive controls, were not reported. No numerical data presented.	Aulicino et al. 1976; Bignami et al. 1977; and Morpugo et al. 1979
		Not given; 0.8 ppm of picloram and 1.5 ppm of Tordon in a nonselective test for nondisjunction.	Negative		
Picloram, Tordon® (99.9%)	Salmonella typhimurium Strains TA1535, TA1536, TA1537 and TA 1538	200 µg/plate	Negative	Salmonella tested in presence and absence of a rat liver S-9 metabolic activation system.	Carere et al. 1978

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Picloram, Tordon (99.9%)	Streptomyces coelicolor	200 µg/plate	Treated plates had approximately 40 times the number of revertant colonies compared to controls.	Hitherto untried system. First time this endpoint was used.	Carere et al. 1978
Picloram	Saccharomyces cerevisial Strains 632/4, 632-1b, 814-18b	1 ppm	Increase in the number of revertant mutants with S. cerevisiae, strain 632/4.	Not statistically significant; probability of error greater than 0.05.	Guerzoni et al. 1976

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Picloram

Formulations	Organisms	Dose	Comments	References
Carboxyl - ^{14}C-labelled picloram	Dog	97 mg/kg (feed) in diet	90 percent of the compound was excreted within 48 hours in the urine. Small amount appeared in the feces.	Redemann 1963 (unpublished GS-609) and McCollister and Leng 1969
	Rat	50 mg/kg in feed	Urine contained ^{14}C only as unchanged picloram. No ^{14}C was detected in exhaled CO_2.	
	Rat	Single dose of 20 mg/kg body weight in feed	^{14}C in urine reached a peak in 2 hrs. and was 100 times blood concentration and 10 times that in stomach. Tissues (liver, thigh muscle, kidneys, abdominal fat) attained maximum levels 2-3 hours after dosing. Levels fell rapidly in 6 hours and could not be detected in 12 hours. At the time of maximum blood level, the concentration in tissues was from 5-6 times that in blood and the stomach had emptied 50% of its picloram content.	Redemann 1964 (unpublished GS-523)
Technical	Cow	5 mg/kg feed for 4 days	97 percent of the ingested dose was accounted for as unchanged compound in the urine.	Fisher et al. 1965
Technical	Sheep	220 mg/kg feed for 1 week	Maximum levels of 0.25 ppm was found in the blood and fell to 0.01 ppm or less within 96 hours after withdrawal of treatment. Peak values of 880 and 350 ppm in two sheep fell to 52 and <1 ppm, respectively.	McCollister and Leng 1969

Appendix F (Continued)

Formulations	Organisms	Dose	Comments	References
Technical (97% pure)	Steers	100, 200 mg/kg feed for 31 days	At different times during feeding period blood samples contained maximum levels of 0.5 ppm at both dietary levels. Although directly proportional to feed levels, none of the tissues (heart, liver, brain, lean muscle, omental fat) from animals sacrified at 31 days had a residue level higher than 0.5 mg/kg. Concentrations in kidneys were 4 and 10 mg/kg in animals fed 100 or 200 mg/kg feed, respectively.	McCollister and Leng 1969
Potassium salt (22.8% acid equivalent)	Dairy Cow	10 to 1,000 mg/kg feed for 6 days to 2 weeks	No measurable residues (<0.05 ppm) were found in milk samples from cow fed 10 to 100 mg picloram/kg feed. Milk samples from cows fed 150 to 1,000 mg picloram/kg feed contained 0.05 to 0.29 ppm residues. Residues declined rapidly and became undetectable 58 hours after treatment was discontinued.	Kutschinski 1969
Potassium salt (22.8% acid equivalent)	Steers	2 to 1,600 mg/kg acid equivalent in feed for up to 8-weeks	Blood levels, directly proportional to dietary concentration, reached maximum within the 2-week feeding period and declined slowly toward end of second week. At autopsy residues in tissues were directly proportional to dietary levels; 0.05 to 0.5 mg/kg in muscle and fat, 0.12 to 2.0 mg/kg in liver and blood, and 2 to 18 mg in kidney. In one animal fed 1,600 mg picloram/kg feed for 4 weeks and sacrificed 3 days after withdrawal from diet, kidney contained residues less than 0.1 mg/kg and other tissues contained less than 0.05 mg/kg residues.	Kutschinski and Riley 1969

Appendix F (Concluded)

Formulations	Organisms	Dose	Comments	References
Picloram, potassium salt (99.6% acid equivalent)	Human Males	Each subject given two oral doses of 0.5 and 5.0 mg/kg and one dermal dose of 2.0 mg/kg at 2-week intervals	Concentrations of picloram measured in blood and urine. Oral dose assimilated rapidly ($t_{1/2}$ = 0.5 hr) and rapidly excreted in urine. >75% excreted within 6 hours, >90% excreted within 72 hours. Dermal dose absorbed slowly ($t_{1/2}$ = 12 hr) and only 0.2% of applied dose was absorbed.	Nolan et al.

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Herbicide Background Statement: Simazine

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Simazine Summary

Major Trade Names: Princep® and Aquazine® in the United States and Princep® , Gesatop® , Primatol S®, Sumanex®, Simazol®, and Framed® in Europe and Canada.

Chemical Name: 2-chloro-4,6-bis (ethylamino)-s-triazine

Major Forestry Application: Selective control of annual and perennial grasses and broadleaved weeds, algae, and aquatic plants.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Backgound Statement.

Toxicology

Simazine is generally nontoxic or only slightly toxic to a variety of nontarget organisms. Studies with invertebrates and microorganisms showed that simazine generally is toxic at concentrations greater than 10 ppm. Investigations in birds and fish have shown low levels of toxicity in these species

The acute oral LD_{50} of simazine in rodents and rabbits is greater than 5,000 mg/kg. Rats fed up to 100 ppm in the diet for two years exhibited no signs of toxicity. Tests on rabbits indicate that simazine is not likely to be absorbed through the skin and poses no real hazard from dermal contact. Toxicity from inhalation is low. Simazine was nonmutagenic when tested in a number of mutagencity tests and only weakly mutagenic in the fruit fly. Simazine was noncarcinogenic in mice fed 603 ppm in the diet for 18 months.

Environmental Fate

Simazine is readily taken up by the roots of plants and translocated to the above-ground portions where it interferes with a variety of biochemical processes. It has a low tendency to bioaccummulate in animals and is rapidly metabolized to nontoxic metabolites that are excreted via the kidneys.

Simazine is moderately persistent in soil, and is primarily lost by means of microbial and chemical degradation. It is resistant to leaching in soil and readily adsorbs to soil particles. In water, simazine is moderately persistent but due to its low water solubility, it is not readily transported in soil run-off to water-bodies.

Exposure and Hazard

Estimated occupational and environmental exposures to simazine from normal use are below those levels that would be expected to have acute or chronic toxic effects or reproductive effects. There should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from USDA Forest Service use of simazine if proper care is taken during application.

1.0 General Information

1.1 Normal Use Patterns

Simazine (also known by the trade names* Princep® and Aquazine® in the United States and Princep®, Gesatop®, Primatol S®, Simanex®, Simazol® and Framed® abroad) is a herbicide used for selective control of annual and perennial grasses and broadleaved weeds. It is effective in controlling aquatic plants and as an algicide in farm ponds, swimming pools, and water cooling towers (Meister Publishing Company 1981 and Weed Science Society of America 1983). The s-triazines, which include both simazine and atrazine, affect many biochemical processes in plants including photosynthesis, plant growth regulation, nitrogen metabolism, and nucleic acid metabolism. Simazine is absorbed primarily through the roots and is translocated upward in the plant stem through the xylem to accumulate in sites of new growth in both stems and leaves (Esser et al. 1975).

Simazine has a large number of herbicidal and algicidal uses. It is registered for use as a weed control agent for a variety of broadleaf and grass weeds among crops such as berries (blueberries, caneberries, blackberries, boysenberries, loganberries, raspberries, cranberries), grapefruit, and oranges (U.S. Department of Energy 1983). In noncrop areas, simazine is used for nonselective weed control in industrial sites, along railroad and highway rights-of-way, and in noncrop farm areas such as nurseries, shelter belts, and christmas tree plantations (Geigy Chemical Corporation 1970, sample label quoted in U.S. Department of Energy 1983). As a result of its low toxicity to many aquatic organisms, especially fish, and its relative noncorrosiveness, simazine is used as an aquatic weed and algae control agent in recirculating water applications such as large aquaria, swimming pools, fountains, and water cooling towers (Weed Science Society of America 1983).

Simazine is used in forestry for site preparation, conifer release, timber management, nursery transplant applications, noxious weed control, wildlife habitat management, and ditchbank vegetation control (Ghassemi et al. 1981 and Gross 1983). Application rates ranged from 2 to 12 lbs. acid equivalent per acre for 18 USDA Forest Service projects in which simazine was used during 1982 (Gross 1983). Simazine is usually applied at a rate per acre of 3 to 4 lbs of 80 percent a.i. in 10 gallons of water for site preparation and conifer release (Ghassemi et al. 1981). For nursery applications,

*Trade names are used only to provide information and do not imply product endorsement.

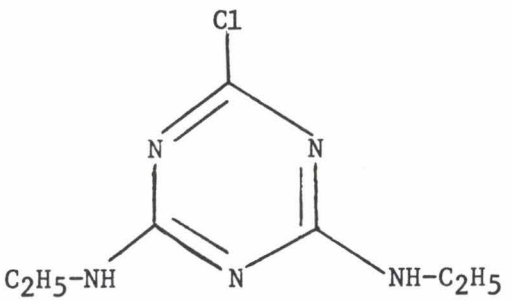
1.6 to 3.8 lbs of 80 percent a.i. in 10 to 25 gallons of water are generally applied. Statistics on the use of simazine by the USDA Forest Service are presented in Table 1-1.

Simazine is manufactured and marketed in the United States by the Ciba-Geigy Corporation under the trade names Princep® and Aquazine® and is available as a wettable powder, as granules, and in liquid suspension form. Abroad, simazine is manufactured and/or marketed under a variety of trade names by several companies including Ciba-Geigy Corporation. Simazine is also variously tank-mixed with atrazine for weed control in corn; with paraquat for use in nut and fruit tree orchards, corn, and grapes; and with amitrole for use around ornamental and Christmas tree plantings (Meister Publishing Company 1981 and Weed Science Society of America 1983). Table 1-2 lists the major formulations of simazine marketed in the United States.

Simazine is effectively applied in late winter, early spring, or early fall, depending upon regional weather patterns. Effective use of simazine requires enough precipitation to permit the material to be leached to plant root zones (Witt and Baumgartner 1979 in Ghassemi et al. 1981). In aquatic applications such as recreational and farm ponds, simazine can be applied from the shore or from several points in the pond from which the chemical is readily dispersed by natural water movement (Weed Science Society of America 1983).

1.2 Physical and Chemical Properties

The active ingredient of simazine and its formulations is 2-chloro-4,6-bis(ethylamino)-s-triazine, with the following structural formula (Esser et al. 1975):



Simazine has a molecular formula of $C_7H_{12}ClN_5$ and a molecular weight of 201.7. Pure simazine is a white crystalline solid with a melting point of 437 to 441°F (225 to 227°C). Simazine is not very soluble in water (3.5 ppm by weight at 68°F [20°C] and 84 ppm by weight at 185°F [85°C]). In chloroform and methanol at 68°F (20°C), simazine solubility is 900 and 400 ppm by weight, respectively, but in other organic solvents such as n-pentane and petroleum ether,

Table 1-1

USDA Forest Service Simazine Use

Purpose	Active Ingredient Per Acre (lbs)		Acres Treated	Formulation	Application Method
	Range	Average			
1981					
Ground					
Aquatic weed control	1.6	1.6	180	Liquid	Broadcast
General weed control	1.2 - 3.0	2.0	51	Liquid	Broadcast
Right-of-way	4.0 - 12.3	12.1	451	Liquid	Broadcast
Site preparation	5.2	5.2	48	Liquid	Spot
1982					
Ground (18 Projects)	2.0 - 12.0	5.0	3329	Granular	Ground Foliar

Source: Gross 1982 and 1983.

Table 1-2

Simazine Formulations

Formulation[a]	Active Ingredients	Comments	Formulator
Princep®	Simazine (2-Chloro-4,6-bis [ethylamino]-s-triazine) in various concentrations	There are various formulations of Princep and Aquazine that differ primarily in terms of percent active ingredient and type of formulation. Specific formulation names include numbers and letters which designate percent active ingredient (simazine) and type of formulation, respectively. Types of formulations are:	Ciba-Geigy Corporation
Aquazine®	Simazine (2-Chloro-4,6-bis [ethylamino]-s-triazine) in various concentrations	W = wettable powder, G = granular, and L = liquid. Thus, Princep® 80W is a formulation of 80 percent active ingredient simazine in a wettable powder.	Ciba-Geigy Corporation

[a] Simazine formulations with other herbicides are not included.

Source: Gosselin et al. 1976.

solubility of simazine is less than in water at comparable temperatures (Esser et al. 1975 and Weed Science Society of America 1983).

Simazine is degraded by nonbiological mechanisms. Jordan et al. (1970) reviewed the processes by which simazine and other s-triazines are degraded. These processes include photodecomposition, volatilization, hydroxylation, and dealkylation. Laboratory studies have shown that exposure to both ultraviolet and infrared light results in loss of herbicidal activity in simazine as a result of photodecomposition. However, in field studies, loss of simazine activity due to photodecomposition has not been adequately demonstrated. Simazine has a low vapor pressure, ranging from 9.2×10^{-10} mmHg at 50°F (10°C) to 9.0×10^{-7} mmHg at 122°F (50°C) (data furnished by Geigy Chemical Corporation in Jordan et al. 1970). At ambient temperatures, only small amounts of simazine vaporize. Simazine is readily hydrolyzed by a nonbiological reaction to form a nonphytotoxic metabolite, hydroxysimazine. Finally, there is evidence that dealkylation by nonbiological free radical reactions in soil environments act to degrade simazine.

The physical and chemical properties of simazine have had a profound influence on the nature of simazine formulations. Specifically, liquid formulations with high concentrations of simazine in water are precluded by the low solubility of simazine. Consequently, wettable powders which also permit ease of transport, measurement, and application have been developed. These powders, in commercial form, contain as much as 80 percent simazine, or mixtures of simazine and other s-triazines (such as atrazine). In addition to simazine, such powders contain wetting agents, dispersing agents, and inert chalk or clay to make up the bulk of the formulation. Granular formulations of simazine permit application without the added bulk and weight of water, and are particularly useful in large-scale ground and aerial applications. Recent development has shown that simazine can be formulated as a fine suspension concentrated in water or oil. These liquid formulations have the advantage of being flowable and readily measured by volumetric means (Esser et al. 1975).

2.0 Toxicity of Simazine

The toxicological properties of simazine, summarized in Table 2-1, show that this herbicide is generally nontoxic or only slightly toxic to a variety of organisms, although a few studies found toxic effects. Its relatively low toxicity to animal species, coupled with a high degree of phytotoxicity, makes simazine a potentially effective herbicide for a variety of applications. However, the low solubility of simazine in water and its toxic effects on nontarget plant species require special techniques in formulation to maintain suitably high effective concentrations for herbicidal use. Special care is also required during application to avoid injury or mortality of nontarget plant species.

2.1 Invertebrates and Microorganisms

Simazine toxicity has been determined in a wide variety of invertebrate species (mostly arthropods) and microorganisms from freshwater, terrestrial, and marine ecosystems. Clark et al. (1970) have proposed that chemical substances with LC_{50}'s greater than 10 ppm in aquatic organisms be considered "slightly toxic," substances with LC_{50}'s between 1 and 10 ppm be considered "toxic," and substances with LC_{50}'s less than 1 ppm be considered "highly toxic." Major findings of the various studies involved are summarized in Appendix A and indicate that, in many of the organisms studied, toxic effects were seen only at concentrations greater than 10.0 ppm, indicating slight toxicity of simazine. In some studies, toxic effects were observed at 1.0 to 10.0 ppm concentrations (toxic levels) and in a very few studies, effects were observed at concentrations less than 1.0 ppm, indicating that simazine was highly toxic in these rare instances. Most of the studies where simazine was shown to have highly toxic effects on aquatic organisms were conducted in field situations. Because of the strong toxic effect of simazine on aquatic plants, the toxic effects observed on aquatic animal species may have been due to oxygen depletion following death of algae and aquatic plants, rather than to direct toxic effects on the species tested.

In evaluating toxicity of simazine to aquatic organisms in natural environments, the maximum solubility of simazine, which is approximately 5.0 ppm at environmental temperatures, should be taken into consideration. Any aquatic toxicity values that are higher than this maximum solubility of 5.0 ppm are a measure of both dissolved and undissolved simazine (Mauck 1974). Most toxicity studies with aquatic organisms, including both fish and invertebrates, indicate that the toxicity of simazine is greater than 5.0 ppm, although the actual concentration of dissolved simazine in water should never exceed 5.0 ppm at temperatures in the range of survival (usually less than 100°F) for aquatic animals. Laboratory toxicity studies have often required the use of suspensions of simazine, such as in the

Table 2-1

Summary of Toxicological Properties of Simazine[a]

1. Acute Oral Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Simazine)	Ingestion of a single dose	LD_{50} >5,000 mg/kg
Mouse (Simazine)	Ingestion of a single dose	LD_{50} >5,000 mg/kg
Rabbit (Simazine)	Ingestion of a single dose	LD_{50} >5,000 mg/kg
Prairie vole (Simazine)	Ingestion of a single dose	LD_{50} = 3,920 mg/kg in male and 3,980 mg/kg in females
Gray-tailed vole (Simazine)	Ingestion of a single dose	LD_{50} = 2,010 mg/kg in males and 2,690 mg/kg in females
Fish (various)	Surface absorption	LD_{50} generally >10 ppm
Chickens (Simazine, 80% wettable powder)	Ingestion of a single dose	LD_{50} > 5,000 mg/kg (no mortality observed)
Pigeons (Simazine, 80% wettable powder)	Ingestion of a single dose	LD_{50} >5,000 mg/kg (no mortality observed)

2. Subchronic Oral Toxicity

Organism (Formulation)	Dose and Length and Treatment	Adverse Effects
Cattle (Simazine 80% wettable powder)	10 mg/kg/day for 10 days	None
	low doses 20 to 50 mg/kg/day over several (6 to 10) days	5 to 21 percent weight loss
	100 mg/kg/day for 7 days	Animals moribund and sacrificed. Congested lungs and kidneys; swollen and friable liver
	500 mg/kg/day for 3 days	11 percent weight loss

Table 2-1 (Continued)

2. Subchronic Oral Toxicity (Concluded)

Organism (Formulation)	Dose and Length and Treatment	Adverse Effects
Sheep (Simazine, 80% wettable powder)	25 mg/kg/day for 10 days	None
	50 mg/kg/day for 31 days	Death with 18% weight loss
	50 mg/kg/day for 10 days	18% weight loss
	100 mg/kg for 10-14 days	Death
	250 mg/kg/day for 3 days	9% weight loss
	250 mg/kg/day for 10 days	Death
	400 mg/kg/day for 9 days	Death

3. Acute Dermal Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (Simazine)	One dose applied to skin	LD_{50} >10,000 mg/kg

4. Dermal Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (Simazine)	Repeated application to skin for 21 days	LD_{50} = 2,000 mg/kg. No substantial skin irritation.

5. Eye Irritation

Organism (Formulation)	Exposure	Adverse Effects
Rabbit (Simazine)	71 mg/kg applied to eye	Transient conjunctivitis, no irritation to iris or cornea.

6. Inhalation Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Simazine 80W, 80% wettable powder)	One hour exposure to dust aerosol concentrations of 1.8 to 4.9 ppm	None

Table 2-1 (Continued)

7. Chronic Toxicity

Organism (Formulation)	Exposure	Adverse Effects
Rat (Simazine)	2-year feeding study. Concentrations up to 100 ppm in diet	None

8. Reproductive and Teratogenic Effects

Organism (Formulation)	Exposure	Adverse Effects
Rat (Simazine)	Inhalation for 1 to 3 hours on days 7 through 14 of gestation. Concentrations ranged from 17 to 317 mg/m^3	None

9. Carcinogenicity

Organism (Formulation)	Exposure	Adverse Effects
Mouse (Fogard S®, 37.5% Simazine and 25% Atrazine)	Total of 0.0065 mg given subcutaneousty over approximately 7 weeks.	Increased incidence of malignant lymphomas.
Mouse (Simazine)	Maximum tolerated doses given: 215 mg/kg from days 7-28 and 603 ppm diet for 18 months.	Noncarcinogenic

10. Mutagenicity

Organism	Formulation	
Salmonella typhimurium	Simazine	
Strains, TA100, TA1535, TA1537, TA1538 tested with and without metabolic activation		Nonmutagenic
S. typhimurium	Princep®	Nonmutagenic
Strains TA98, TA100, TA1535, TA1537, TA1538 tested with and without metabolic activation		

Table 2-1 (Concluded)

10. Mutagenicity (Concluded)

Organism	Formulation	Adverse Effects
S. typhimurium Strains TA98, TA100, TA1535, TA1537, TA1538, tested with and without metabolic activation	Princep® + Atrazine	Nonmutagenic
Escherichia coli	Simazine	Nonmutagenic
Bacillus subtilis	Simazine	Nonmutagenic
Saccharomyces cerevisiae	Simazine	Nonmutagenic
Drosophila melanogaster	Simazine	Weakly mutagenic
D. melanogaster	Princep® 80W	Increase in dominant lethals
Serratia marcescens	Simazine	Nonmutagenic
WI 38 cells	Simazine	No DNA damage i.e., no unscheduled DNA synthesis

[a]Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

form of wettable powders. In field situations, it is highly unlikely that such suspensions of high concentrations of simazine in water would persist for any appreciable length of time following normal application.

For the most part, toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results of such studies do not necessarily present a true reflection of the potential toxic effects of simazine in a natural environment. For example, in the laboratory, aquatic organisms were generally kept up to 4 days or more in static conditions (in tanks or in aquaria without a flow-through replenishment of freshwater and/or herbicide). Microorganisms were generally grown in culture media containing various concentrations of simazine. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to simazine or to other man-made chemicals is usually a transient phenomenon depending on streamflow, chemical dissipation, and decomposition. Additionally, in natural systems, interactions with other man-made or naturally occurring chemicals, as well as changes in environmental factors such as temperature and moisture, all potentially affect herbicide assimilation and metabolism by invertebrates and microorganisms and thus alter their tolerance levels.

Insect studies with simazine summarized in Appendix A report simazine effects ranging from LC_{50}'s of 1.9 ppm (Johnson and Finley 1980) to 50 ppm (U.S. Department of the Interior 1968 in Pimentel 1971). Both extremes were for stoneflies. Other studies of groups of insects or groupings of various soil arthropods found population reductions of 50 percent or more with simazine applications as low as 0.5 ppm (Walker 1962 and Pimentel 1971) and as high as 28 ppm (Walker 1964 in Pimentel 1971 and in Mauck 1974). In terms of nonlethal effects, emergence of midge larvae was temporarily delayed at concentrations of 0.66 and 2.2 ppm (studies reported in Ghassemi et al. 1981) and soil arthropod populations were reduced when simazine was applied to field plots, twice each year, at a rate of 1.5 lb/acre (1.68 kg/ha) (Edwards and Stafford 1979). Although specific data are not provided, several sources claim that simazine is not harmful to honeybees (Ghassemi et al. 1981 and Weed Science Society of America 1983).

Numerous studies have shown a wide range of toxicity values for simazine in freshwater and marine crustaceans, ranging from 50 percent mortality in freshwater zooplankton exposed to 1.0 ppm simazine for 24 hours (George et al. 1982) to no observable effects at 1,000 ppm for the mud crab (U.S. Environmental Protection Agency registration data reported by Ghassemi et al. 1981). Within this range of toxicity

values, simazine generally had LC_{50}'s or no observable effect levels (NOEL's) in excess of 10 ppm in most studies, indicating that simazine is only slightly toxic to crustaceans. Indeed, in some organisms studied, such as sowbugs, glass shrimp, and crayfish, LC_{50}'s were found to be in excess of 100 ppm (Sanders 1970, Johnson and Finley 1980).

Some nonlethal effects of simazine were observed in crustacea at relatively low doses. Fitzmayer et al. (1982) found interference with molting and reduced maturation in cladocerans (Daphnia sp.) at 4 mg and 20 ppm simazine (Aquazine®). They also found that reproductive maturation was delayed and both numbers of live young and numbers of broods were reduced at 4 ppm. Reproductive maturation was totally inhibited at 20 ppm. At 0.25 to 3.0 ppm simazine with Daphnia, however, Meyer and Sanders (1977 in Ghassemi et al. 1981) observed no effects on reproduction.

In invertebrates other than arthropods (i.e. in snails, oysters, and worms), the few studies with simazine generally indicate toxic effects at relatively low concentrations, particularly in field studies. Harmon (1978 in Buikema 1981) observed a decline in snail populations in a lake with initial simazine concentrations of 0.5 ppm. In a laboratory test, however, the same snail species tolerated a simazine concentration of 5.0 ppm for 96 hours, leading Harmon (1978 in Bukemia 1981) to conclude that mortality observed in the field populations may have been due to a synergistic effect between herbicide effects and death of algae in the lake. In oysters, no inhibition of shell growth was reported at a simazine concentration of 1.0 ppm (U.S. Environmental Protection Agency registration data reported in Ghassemi et al. 1981). Ghassemi et al. (1981) also reported a study in which populations of terrestrial worms (round worms and segmented earthworms) and mites were inhibited in some habitats, and showed increases in other habitats, when simazine was applied at rates equivalent to 4.5 and 6.7 lb/acre (5.0 and 7.5 kg/ha). DeVries (1962 in Pimentel 1971) exposed worms in potted soil to simazine at doses equivalent to application rates of 3 and 12 lb/acre (3.4 and 13.4 kg/ha) for 32 days and found no mortality in one species (Helodrilus, sp.) and only 1 of 8 earthworms (Lumbricus, sp.) dead at each dose. Walker (1962 and 1964 in Pimentel 1971) reported a population reduction of 50 percent or more in aquatic worms, leeches, and snails after simazine applications of 0.5 to 10.0 ppm, and a 50 percent population reduction in aquatic worms after 96 hours exposure to 28 ppm simazine. Rao and Dad (1979) found a reduction in the rate of asexual budding in colonial freshwater bryozoans exposed to simazine at 0.01 to 1.5 ppm concentration.

A variety of studies have examined the effects of simazine on soil microorganisms and fungi in the field, in culture media, and in potted soil. Applications of simazine at rates up to 3.6 lb/acre

(4 kg/ha) (Bopaiah and Rai 1979) and at concentrations up to 10.0 ppm (Navyar et al. 1970 in Ghassemi et al. 1981) usually resulted in either no effect or in stimulation of growth in soil microorganism populations. The activity of the enzyme urease, however, was inhibited when soil microorganisms were exposed to simazine at a concentration equivalent to an application rate of 8.9 lb/acre (10 kg/ha) (Manorik and Malichenko 1969 in Grossbard and Davies 1976). Furthermore, nitrification was inhibited at concentrations of 6 ppm (quoted by Audus 1970 in Grossbard and Davies 1976). On the other hand, Kaufman et al. (1965) found that the fungus Aspergillus fumigatus was capable of metabolizing the carbon in simazine when exposed at simazine concentrations of 5 ppm in culture medium.

2.2 Fish

The toxic effects of simazine and its formulations on fish have been reported in numerous studies, which are summarized in Appendix B. In general, simazine is only slightly toxic to fish. Toxicities range from a 96-hour LC_{50} of 0.25 ppm in striped bass (Wellborn 1969 in McCann and Hitch 1980), in a study that McCann and Hitch (1980) were unable to reproduce, to a 96-hour LC_{50} greater than 1,000 ppm for both bluegill and channel catfish, reported by Ghassemi et al. (1981) from U.S. Environmental Protection Agency registration data. Table 2-2 indicates the extreme median lethal concentrations of simazine as reported for four selected fish species and indicates a wide variability in values for each of the species listed. As indicated in Appendix B, however, most studies of simazine toxicity to fish report LC_{50}'s greater than 10 ppm, indicating slight toxicity of simazine to fish. Many of these studies report LC_{50}'s in excess of 100 ppm, which, when viewed in context of the solubility of simazine in water at normal environmental temperatures (5 ppm), indicates that normal applications of simazine should have little or no adverse effect on fish populations (although few NOEL's for fish are available).

Although most fish are relatively resistant to simazine concentrations up to 10 ppm, several studies have reported various sublethal effects of simazine. Dodson and Mayfield (1979) exposed rainbow trout to technical grade simazine and Tween 80®, a wetting agent, and found that the fish exhibited a decreased swimming speed and a decreased swimming response to currents at simazine concentrations of 1.0 to 12.5 ppm after 24 hours exposure. However, these authors found that when trout were exposed to Princep® 80W at these same concentrations but without the Tween 80®, there was no impairment of swimming speed or response to currents, indicating that the toxic behavioral effect was probably a result of exposure to the Tween 80® wetting agent rather than to the simazine. Fish ponds, stocked with

Table 2-2

Extreme Median Lethal Concentrations of Simazine
for Selected Fish Species

Fish Species	Observed Median Lethal Concentrations (LC_{50})		References[b]
	Lowest	Highest	
Striped bass	0.25 ppm (96-hour)[a]	>180 ppm (96-hour)	Wellborn 1969 in McCann and Hitch 1980; McCann and Hitch 1980
Rainbow trout	2.8 ppm (96-hour)	NOEL at 200 ppm (24-hour)	Ciba-Geigy test data in Mauck 1974; Dodson and Mayfield 1979
Bluegill	11.6 ppm (96-hour)	>1,000 ppm (96-hour)	Cope 1965 in Mauck 1974; U.S. Environmental Protection Agency registration data cited by Ghassemi et al. 1981
Channel catfish	65.0 ppm (96-hour)	>1,000 ppm (96-hour)	Bathe et al. 1974; U.S. Environmental Protection Agency registration data cited by Ghassemi et al. 1981

[a] LC_{50} of 0.25 ppm for striped bass reported by Wellborn (1969) but could not be repeated by McCann (1980).

[b] First reference is the source for the lowest LC_{50}, second reference is the source for the highest LC_{50}.

channel catfish after treating the pond bottoms with 12 lbs. Aquazine® a.i./acre (13.4 kg/ha) one day prior to filling with water, yielded 19 percent less fish biomass than controls after six months (Tucker and Boyd 1978). A single application of Aquazine®, to give a concentration of 1.5 ppm in fertilized bluegill ponds, resulted in an 11 percent reduction in yield after 128 days (Tucker and Boyd 1978). Rao and Dad (1979) reported an increase in the rate of opercular beating (beating of the gill covers) in three species of Indian teleost fishes with increasing concentrations (not specified) of simazine during a 96-hour static bioassay. Dad and Tripathi (1980) reported general responses of fish to toxic stress from simazine including loss of equilibrium, increased mucus secretion, increased rate of excretion, and loss of pigment at higher toxic concentrations (not specified).

2.3 Birds

Dietary studies and egg injection bioassays with simazine are summarized in Appendix C and indicate that simazine has a low toxicity to birds as tested. Adverse effects ranged from reduced weight gain in chickens dosed at 50 mg/kg for 10 days by capsule (Palmer and Radeleff 1969) to an LC_{50} of 51,200 ppm in a dietary study reported by Ciba-Geigy Chemical Corporation (1970 in Mauck 1974).

In general, dietary studies indicated a low toxicity of simazine to bird species. Five-day feeding studies resulted in an LC_{50} >5,000 ppm for bobwhite, ring-necked pheasant, and mallards and an LC_{50} >3,720 ppm for Japanese quail fed 99.1 percent technical simazine (Hill et al. 1975). Data provided by Ciba-Geigy Chemical Corporation (1970 in Mauck 1974) are generally in agreement with Hill et al. (1975), with the exception of a higher LC_{50} of 51,200 ppm for mallards in the Ciba-Geigy data. Palmer and Radeleff (1969) found that 10-day ingestion of 50 mg/kg body weight resulted in a decrease in weight gain in dosed chickens compared to controls.

In a reproductive study with chickens, Fink (1975 in Ghassemi et al. 1981) found that dietary levels of 2.0 and 20.0 ppm, both before and during normal egg production cycles, did not affect reproductive function. In egg injection studies, Dunachie and Fletcher (1970) reported a decreased proportion of hatching compared to controls (94 percent of controls) at doses giving a concentration of 300 ppm in the egg. No effects in terms of hatchability were noted at 200, 100, or 10 ppm simazine in the eggs.

2.4 Mammals

The potential health hazards from exposure to simazine have been evaluated in laboratory animals such as mice, rats, rabbits, and dogs as well as in domesticated animals such as sheep and cattle. Studies

in wildlife animals have been restricted to microtine rodents (voles). Simazine generally has low toxicity for these species; the acute toxicity following ingestion is greater than 2,000 mg/kg in voles and greater than 5,000 mg/kg in the other species. The results of the various toxicity tests are described below and are summarized in Table 2-1 and Appendix D and E.

2.4.1 Acute Toxicity

Acute toxicity data have been reported for acute oral, dermal, eye irritation, and inhalation studies.

2.4.1.1 Oral. The acute oral toxicity of simazine to mammals is very low. Following ingestion of a single dose of simazine, the LD_{50} for rats, mice, and rabbits was found to be greater than 5,000 mg/kg (U.S. Department of Agriculture 1981). The acute oral LD_{50} was found to vary from approximately 2,000 mg/kg in gray-tailed voles to over 3,900 mg/kg in prairie voles. These toxicity values in experimental laboratory animals place simazine into toxicity category IV, warranting handling with caution, according to U.S. Environmental Protection Agency toxicology guidelines (see Table 2-1 in the General Introduction to the Herbicide Background Statements).

2.4.1.2 Dermal. When applied to the skin of rabbits, the dermal LD_{50} for simazine was reported to be greater than 10,000 mg/kg. These results indicate apparent lack of absorption of the material through intact skin or absence of toxicity and show that simazine poses no real hazard from dermal contact.

2.4.1.3 Primary Eye Irritation. When applied to the eye of rabbits, simazine produced only a transient inflammation of the conjunctiva but no irritation to the iris or cornea (U.S. Department of Agriculture 1981).

2.4.1.4 Inhalation. In an inhalation study, rats were exposed for one hour to dust aerosols of Simazine 80W in concentrations ranging from 1.8 to 4.9 ppm of atmosphere. No deaths or signs of other toxicological or pharmaceutical effects were reported (Weed Science Society of America 1983).

2.4.2 Subchronic Toxicity

Subchronic toxicity data have been reported for oral and dermal studies.

2.4.2.1 Oral. Results of subchronic oral toxicity tests of simazine in cattle and sheep have been reported by Palmer and Radeleff (1969). In these studies, poisoning was considered to have occurred when any sign of abnormal function or behavior was observed. These

signs ranged from loss of appetite, depression, muscular spasms, dyspnea, weakness, uncoordinated gait, and moribundity or death. A weight loss of 5 percent or more was considered significant.

Cattle treated with simazine at the rate of 10 mg (a.i.)/kg body weight showed no ill effects following 10 daily doses by drench. Sheep similarly treated with 25 mg (a.i.)/kg per day for 10 days showed no ill effects.

Higher doses produced various signs of toxicity in both cattle and sheep. Cattle treated with simazine at exposures ranging from 20 mg/kg per day for 6 doses to 50 mg/kg per day for 10 doses showed signs of poisoning, and weight loss ranged from 5 to 21 percent depending upon the dosing schedule. Cattle treated with 7 doses of 100 mg/kg day became moribund and were sacrificed. Necropsy showed congestion of lungs and kidneys, swollen, friable livers, and small hemorrhagic spots on the surface of the lining of the heart. Cattle, administered 250 mg simazine per kg body weight for 3 days, showed signs of intoxication but survived with an 11 percent weight loss.

Sheep treated with 50 mg simazine/kg body weight for 10 days showed signs of intoxication but survived with an 8 percent weight loss. Sheep dosed with 100 mg simazine/kg died after 14 doses by drench or 10 doses by capsule. The latter showed a 13 percent weight loss. Sheep treated with 250 mg/kg showed signs of poisoning after one dose but survived with 9 percent weight loss. However, animals given 250 mg/kg per day for 10 doses by capsule or 400 mg/kg for 9 doses by drench were poisoned and died.

2.4.2.2 Dermal Irritation. In a 21-day study in which simazine was applied to the skin of albino rabbits, the dermal LD_{50} was 2,000 mg/kg (U.S. Department of Agriculture 1981). No signs of skin irritation were reported when 200 mg/kg were applied.

2.4.3 Chronic Toxicity

In a two-year chronic oral feeding study, rats of both sexes were given diets containing Simazine 50W in concentrations as high as 100 ppm. No gross or microscopic signs of toxicity were reported (U.S. Department of Agriculture 1981 and Weed Science Society of America 1983).

2.4.4 Reproductive and Teratogenic Effects

The evaluation of the reproductive and teratogenic effects of simazine are limited to a study in which simazine was administered by inhalation (Newell and Dilley 1978). In this study, pregnant rats were exposed to concentrations of 17, 77, and 317 mg simazine per m^3 atmosphere. The animals were exposed for 1 to 3 hours on days

7 through 14 of gestation. No treatment related teratogenic effects were observed. No other studies on the reproductive effects of simazine have been reported in the open literature.

2.4.5 Carcinogenicity

Except for a single report on the carcinogenic potential of simazine alone (Innes et al. 1969) and the formulation Fogard S® (37.5 percent simazine and 25 percent atrazine) (Donna et al. 1981), information regarding the carcinogenic potential of simazine is not available. In a long-term bioassay, simazine was administered to male and female mice of two hybrid strains [(C57BL/6 x C3H/Anf)F_1 and (C57BL/6 x AKR)F_1] for 18 months. In this study, mice were given the maximum tolerated dose of simazine. From days 7-28 of age, mice were treated by stomach tube with 215 mg/kg. Simazine was then mixed in the diet at 603 ppm for the remainder of the experiment. Gross necropsy and histological examination revealed no significant increase in tumor induction related to treatment with simazine.

In the preliminary study of Donna et al. (1981), a group of 25 mice were administered 13 subcutaneous injections of Fogard S® at 3-day intervals for a total of 0.0065 mg active ingredient. The individual doses were equivalent to 0.2 mg a.i./kg/body weight or 0.12 mg simazine/kg body weight. A second group of 25 mice were treated similarly but by the intraperitoneal route. The animals were observed for 7 months, at the end of which time survivors were sacrificed and examined histologically. Of the animals which were treated subcutaneously with Fogard S® and died during the study, 3 of 24 died with malignant lymphomas (cancer of the lymphatic system) and one animal had a peritoneal mesothelioma (a tumor arising from the cells of membranes lining the abdominal cavity). In the group treated intraperitoneally with Fogard S®, five animals were lost to cannibalism. In the remaining survivors, two spontaneous deaths were atributable to malignant lymphomas. No tumors were found in 100 saline treated control animals. This limited preliminary study appears to indicate that Fogard S® has the potential to induce malignant tumors in mice. The role of simazine alone in this study is not known. In view of the small number of animals and short observation period the results are questionable. Moreover, the chronic long-term study by Innes et al. (1969), in which mice were treated for about 18 months with simazine at a dietary level of 803 ppm (equivalent to approximately 90 mg/kg/body weight) without any significant increases in tumor incidences, indicates that simazine lacks any carcinogenic potential.

2.5 Mutagenicity

Simazine has been evaluated in a variety of microbial mutagenicity test systems which have given negative results. These

include systems employing Salmonella typhimurium (Simmon 1978; Eisenbeis et al. 1981; Commoner 1976), Escherichia coli (Simmon 1978; Fahrig 1974), Bacillus subtilis (Simmon 1978), Serratia marcescens (Fahrig 1974), and Saccharomyces cerevisiae (Simmon 1978).

Simazine did induce DNA damage, indicated by the ability to induce unscheduled DNA synthesis in human WI 38 embryonic lung cells (Simmon 1978).

The only evidence for possible mutagenicity of simazine comes from results with the fruit fly, Drosophila melanogaster. Valencia (1981) reported that simazine was weakly mutagenic in this species. Male flies were exposed by feeding concentrations of 3, 5 and 2,000 ppm simazine in the food. An increase in the frequency of lethal mutations was observed in the sex-linked recessive lethal test.

Murnik and Nash (1977) reported on assays for dominant lethal mutations; sex-linked recessive lethal mutations; and chromosomal breakage, nondisjunction, and loss performed on D. melanogaster males treated by injection or by larval feeding of simazine, atrazine, or cyanazine. All of the herbicides significantly increased the rate of apparent dominant lethals, which the authors thought was due to physiologic toxicity to sperm. Simazine elevated X-linked lethals when injected, but not when larvae were fed simazine in the diet. None of the herbicides significantly increased other chromosomal aberrations such as the partial loss of the Y chromosome or sex chromosome nondisjunction.

It appears from the available data summarized here that simazine is probably only weakly mutagenic.

3.0 Environmental Fate

Simazine is an effective herbicide for a variety of annual and perennial grass species and for shallow-rooted broadleaf plants. Simazine tends to be toxic to nontarget plant species but, due to its low mobility in soil, it is not readily leached into deeper soil zones, and therefore is not as effective in killing deep-rooted plants (Ghassemi et al. 1981). It is moderately persistent in soil, giving full season control (Ashton 1982), is moderately persistent in water (Ghassemi et al. 1981), and has a relatively low toxicity to most animal species. It is absorbed primarily by plant roots, from which it is readily translocated to all of the above-ground parts of the plant with some accumulation at the margins of leaves (Esser et al. 1975). Simazine is readily metabolized in plants by a variety of different metabolic pathways. Simazine is rapidly metabolized and does not bioaccumulate in animals (Witt and Baumgartner 1979 in Ghassemi et al. 1981).

3.1 Bioaccumulation and Metabolism

Bioaccumulation and metabolism of simazine in both animals and plants have been extensively reviewed by Esser et al. (1975). Plant metabolism of simazine is also reviewed by Ghassemi et al. (1981) and by Ebert and Dumford (1976). A number of studies, reviewed in Section 3.1.2, examine the bioaccumulation and metabolism of simazine in animals. A detailed review by Mauck (1974) draws to some extent on data provided by the Ciba-Geigy Chemical Corporation, which were not available to this study as a primary source.

3.1.1 Plants

The major pathway for uptake of simazine is through the roots, with both susceptible and resistant plants readily absorbing the compound. Simazine applied to plant foliage does not penetrate readily, although the addition of an adjuvant, such as mineral oil, tends to increase the rate of foliar penetration. Studies with radiolabeled simazine have shown that once the herbicide is absorbed into the plant roots, it is rapidly translocated by the xylem into the above-ground portions of the plant. It is then distributed throughout the plant, with some tendency for accumulation in the leaf margins. The rates of uptake and translocation appear to be increased by factors such as higher temperature and lower relative humidity, which tend to increase the rate of transpiration and fluid movement within the plant. The increased movement of water thus facilitates movement of simazine into, and through, the plant (Esser et al. 1975).

Phytotoxicity of herbicides is generally due to inhibition of, or interference with, one or more plant biochemical pathways.

Simazine acts on a variety of biochemical processes in plants, including photosynthesis, plant growth regulation, nitrogen metabolism, and nucleic acid metabolism (Esser et al. 1975).

Simazine interference with photosynthesis involves inhibition of the Hill reaction, the reaction in which oxygen is evolved from water in the presence of chloroplasts. The impairment of photosynthesis has several indirect effects, such as closing of stomata (openings in the leaves through which gases pass) as a result of increased carbon dioxide. The closed stomata in turn lead to a decreased rate of transpiration and consequent reduction in water uptake by the roots.

Although the exact biochemical events involved are unclear, simazine, as well as other triazines, appears to act on metabolism of plant hormones, particularly plant growth regulation hormones. This conclusion results from studies in which triazines (including simazine), when applied at low rates, resulted in more vigorous and healthy plants than controls, yet at higher rates of application, resulted in growth inhibition. The enhancement and inhibition of plant growth at different levels of application has been shown to occur in a wide variety of plant species, including both resistant and susceptible plants.

Nitrogen metabolism is influenced by sublethal levels of triazine, resulting in increased protein fractions and dry matter of seeds from some treated plant species. Field and laboratory studies indicate that applications of triazines result in increased uptake of mineral nutrients, including nitrogen (when supplied as nitrates but not when supplied as ammonium). The increased uptake may be due to membrane changes facilitating more rapid uptake, or to larger roots with greater absorptive surface area in sublethally treated plants. Finally, there has been some consideration of the possible effect of triazines on nucleic acid metabolism, since nucleic acid metabolism is closely linked with growth and with protein metabolism. Experimental results are such that it is difficult to interpret the potential influence, if any, on plant nucleic acid metabolism.

The ability of any given plant species or variety to resist herbicidal activity of simazine is directly related to its ability to degrade simazine, hence those species or varieties which readily degrade simazine to nonphytotoxic or less phytotoxic compounds are less susceptible to its herbicidal activity. In plants, at least six types of reactions have been identified by which simazine can be biodegraded, including hydrolysis, conjugation of simazine with other molecules in the plant, removal of side chains from the simazine molecule with liberation of carbon dioxide, modification of the side chain of the simazine molecule by processes such as oxidation, replacement of amino groups (NH_2) by hydroxy (OH) substituents, and cleavage of the triazine ring structure with ultimate production of

carbon dioxide (Esser et al. 1975). Studies with various chloro-triazines (of which simazine is one) in living plants indicate that "hydroxytriazines" formed by hydrolysis of the parent triazine are primary degradation products.

3.1.2 Animals

Simazine has a low tendency to bioaccumulate in animals, and once absorbed by animals it is rapidly metabolized to nontoxic metabolites that are readily excreted via the kidneys. Appendix F summarizes studies on the bioaccumulation and metabolism of simazine. A detailed review of the metabolism of simazine, as well as the other triazine herbicides, can be found in Esser et al. (1975).

Fish exposed to simazine for as long as 30 days generally accumulated simazine rapidly, with tissue residue levels reaching a steady-state within one or two days. Concentrations of simazine in fish exposed to the herbicide are generally lower than, but proportional to, the environmental concentrations. This phenomenon of a lower tissue concentration is likely a result of the lower solubility of simazine in organic solvents than in water. In laboratory studies, when fish that had been exposed to simazine were placed in fresh (simazine-free) water, the fish were rapidly cleared of simazine residues (Bathe et al. 1975; Dodson and Mayfield 1979; and Mauck 1974).

Simazine degradation in animals has been shown to occur by several pathways and has been postulated to occur by several others (Esser et al. 1975). These pathways include hydrolysis of the parent simazine to hydroxysimazine, conjugation (combining) of the parent herbicide with other organic metabolites in the animal, removal of the side chains and their further degradation to carbon dioxide, modifications to the side chains, and cleavage of the triazine ring. Hydrolysis of simazine to hydroxysimazine has been demonstrated in rats using ^{14}C-labeled simazine. Hydroxysimazine is readily eliminated from rats both in urine and in feces, with urine being the primary route of elimination. Conjugation does not appear to have been reported with simazine, although conjugation of another triazine (cyanazine) with an amine acid has been demonstrated in animals. Using simazine with ^{14}C-labeled side chains, rapid degradation of 20 to 75 percent of the applied chemical was demonstrated in rats as a result of side-chain degradation. These degradation products are excreted in the urine. Although some triazines have been shown to undergo degradation by ring cleavage with subsequent carbon dioxide production, this process is not thought to be an important route of triazine degradation in animals and does not appear to have been observed with simazine.

In general, when simazine is ingested by animals, it is degraded into intermediate metabolites that are readily excreted by

the kidneys. When plant metabolites are fed to animals, they are further metabolized by the animals. Studies with ^{14}C-labeled triazines indicated rapid and complete elimination of radioactivity, primarily via urine. Various studies indicate that only a small amount of the parent simazine is excreted via the feces, and generally only simazine metabolites are excreted in the urine. Generally, triazines are excreted very rapidly, with 50 percent of the applied dose eliminated within 12 to 26 hours in laboratory animals and 1 to 2 days in ruminants. When goats and cows are given single doses of triazine at a rate of 1 mg/kg, transient residues occur in the milk, with peak concentrations 8 to 24 hours after ingestion and rapid decline in residues to the limit of detection within 2 to 4 days (Esser et al. 1975). However, there are few studies on simazine metabolism in the open literature.

3.2 Soils

The fate and transport of simazine in soil has been reviewed by a number of authors, including Esser et al. (1975) and Ghassemi et al. (1981). In addition, Volume 32 of Residue Reviews is a compilation of 14 papers on the fate and transport of triazines in the soil environment by Bailey and White (1970), Behrens (1970), Dubach (1970), Gast (1970), Hayes (1970), Helling (1970), Jordan et al. (1970), Kaiser et al. (1970), Kaufmann and Kearney (1970), Knusli (1970), LeBaron (1970), Mattson et al. (1970), Sheets (1970), and Weber (1970). These papers are listed in the reference section.

Simazine is moderately persistent (3 to 12 months) in soil compared to other herbicides, permitting full season control of vegetation (Ashton 1982). Simazine has a relatively low solubility in water (Weed Science Society of America 1983), has a moderate (Helling 1970, 1971) mobility in silty clay loam, and is relatively readily adsorbed onto soil particles, particularly in muck and clay soils with high organic content (Weed Science Society of America 1983). Chemical and microbial degradation are the primary means by which simazine is lost from soil environments. In field situations, particularly in forest environments, photodegradation and volatilization should not account for any significant loss of simazine (Ghassemi et al. 1981 and Weed Science Society of America 1983).

The persistence of simazine has been studied in a wide variety of soil types in North America and Europe. Simazine's moderate persistence under most conditions provides residual activity that makes it particularly useful for weed control and soil sterilization applications. At the same time, however, this persistence can result in injury to plants such as sensitive rotational crops (Sheets 1970). In an early review of soil persistence of triazines, Sheets (1970) reported that most studies found simazine to be more persistent than

atrazine, that it persisted longer in soils with neutral or high pH than those with low pH, that it was more readily lost during warmer weather and higher precipitation, and that the rate of disappearance of simazine from soil is directly proportional to its concentration. Table 3-1 summarizes major studies on simazine persistence in soil conducted in the United Kingdom and in North America during the past 10 years. These studies confirm that simazine has a moderate persistence which varies according to application rates and environmental conditions, and that simazine tends to remain in the top portions of the soil. Of particular interest is the study by Khan and Marriage (1979) in which only simazine and its metabolite hydroxysimazine were detected up to 40 months after simazine was applied at a rate of 4 lb. a.i./acre (4.5 kg/ha). The greater proportion of hydroxysimazine (40 times that of simazine) indicates degradation of simazine to hydroxysimazine as well as the relatively long persistence and low rate of degradation of hydroxysimazine.

Helling (1970, 1971) compared simazine to other triazines and to 39 other herbicides in the laboratory and demonstrated that, in silty clay loam, simazine was only moderately mobile. In a comparative study with simazine and terbacil, Jensen and Kimball (1982) found simazine to be less mobile than terbacil. One year after application of 2.7 lb/acre (3 kg/ha), simazine residues were found to decrease with depth and were 0.005 ppm in the 15 to 25 cm. layer compared to 0.05 to 0.06 ppm in the top 5 cm. Lavy (1970) demonstrated that simazine has a lower diffusion rate in soil than atrazine and that increased soil moisture, increased soil pH, and increased temperature resulted in an increased rate of diffusion of simazine in soil.

Simazine readily adsorbs to soil particles and is relatively resistant to leaching (Weed Science Society of America 1983). The adsorption of simazine to soil is greatly increased in soils with a high organic content. Since forest soils have large amounts of organic matter, they should offer a high degree of resistance to leaching of simazine. Hance et al. (1976) concluded that downward movement of simazine in soil was more a function of mechanical movement of soil particles (with adsorbed simazine) or of the activities of soil macrofauna than of leaching, since 93 percent of the simazine recovered 2 years after application was found in the top 2 inches (5.1 cm) of the soil profile.

Microbial degradation of simazine has been variously reviewed by Esser et al. (1975), Ghassemi et al. (1981), and Kaufman and Kearney (1970). There is a large body of literature indicating that soil microorganisms are capable of metabolizing triazines in general, and simazine in particular, as a source of energy. Kaufman and Kearney

Table 3-1

Summary of Soil Persistence Studies with Simazine

Formulation and Application Rates	Effects	Comments	References
Simazine, 4 lb/acre (4.5 kg/ha) applied in late May (Ohio)	Degraded through 3 half-lives within 160 days.	Under both no-till and conventional management.	Nearpass et al. 1978
Simazine, 1.0 to 7.9 lb/acre (1.1 to to 8.8 kg/ha) annual dose, 5 to 9 years treatment, various sites in United Kingdom	Residues from "normal" application rates (1.1 to 2.75 kg/ha) were always less than 31% of annual dose, at most sites were less than 10% of annual dose. Most of residues in top 4 inches (10 cm) of soil.	Samples taken in winter, simazine applied either in spring or in spring and late summer. Fruit, ornamental, and forestry crops.	Clay 1978
Simazine, 4 lb/acre (4.5 kg/ha) applied in late May for 7 to 9 years (Southern Ontario)	Simazine and hydroxysimazine persisted for 28 to 40 months without progressive accumulation. Hydroxysimazine residues 40 times greater than simazine at 28 or 40 months. Residues primarily in the top 12 inches (30 cm) of soil.	Treated as a peach orchard, then trees removed and soil disked.	Khan and Marriage 1979 (See also Marriage et al. 1975)
Simazine, 2.7 lb/acre (3.0 kg/ha) applied once in mid-September (Nova Scotia)	Total residues in upper 10 inches (25 cm) of soil were 5% or less of applied simazine.	Vegetation removed prior to simazine application, paraquat used to control subsequent growth.	Jensen and Kimball 1982
Simazine, 0 to 16 lb/acre (0 to 18 kg/ha) applied once as a granular formulation in fall (Vermont)	Weed control effects and fir responses still evident at 4th year, but simazine residues less than 0.1 ppm to depths up to 1 ft (30.5 cm) by 8th year.	Weed control in young Fraser fir Christmas tree planation.	Flanagan and McCormack 1981

Table 3-1 (Concluded)

Formulation and Application Rates	Effects	Comments	References
Simazine, 2.5 to 2.0 lb/acre (2.8 to 22.4 kg/ha), 2 to 5 annual treatments in spring, (United Kingdom)	Residues of 0.03 to 0.07 kg/ha 1 year after dose of 2.8 kg/ha; 0.41 kg/ha 8 months after dose of 5.6 kg/ha; 1.09 to 2.34 kg/ha 2.5 years after dose of 22.4 kg/ha; and 1.70 kg/ha 3.5 years after dose of 22.4 kg/ha.	Higher doses resulted in deeper soil penetration of simazine.	Clay and Stott 1973
Simazine, 100 lb/acre (112 kg/ha) once in spring (United Kingdom)	Mean residues sampled up to 5 years after application. At 5 years, mean residues were 14.8 kg/ha, and were primarily (93%) in the top 5 cm. Simazine half-life about 18 months.	A large variation in sampled residue levels was observed.	Hance et al. 1976
Simazine, 3.2 to 25.6 lb/acre (3.6 to 28.7 kg/ha) for 9 years in January or February (California)	21 months after 9th application, total residues of 0.88 to 7.96 lbs/acre. Maximum residues of 0.39 to 3.75 ppm in top 6 inches of soil. No residues at 24 inches or below.	Applied to grapevine rows.	Leonard et al. 1975
Simazine, 4 lb/acre (4.5 kg/ha) (California)	At most sites, phytotoxic effects declined after 12 to 18 months, simazine residues persisted "well over" 2 years.	Effects of organic matter not as marked as with some other herbicides.	Lange et al. 1968
Simazine (50% a.i. wettable powder)	Half-life of simazine was 20 to 44 days (25°C) and was negatively correlated with soil pH.	Laboratory study.	Walker and Thompson 1977
Simazine, 4 lb/acre (4.5 kg/ha) applied in May and in October (Canadian Prairie soils)	When sampled the following May or October (6 months after application) 67 to 89% of the applied simazine remained in the top 5 cm of soil after winter and 14 to 60% after summer exposure.	Generally, carry-over was greatest on silty clay. Negligible residues recovered below 5 cm.	Smith 1975

(1970) list 1 species of actinomycetes, 8 bacteria, and 22 fungi that have been shown effective in metabolizing simazine. Ghassemi et al. (1981) also list an additional 6 species of bacteria and 4 species of fungi that degrade simazine in soil environment. Srirama Raju and Rangaswami (1972) reported that some bacteria and fungi were resistant to both atrazine and simazine at herbicide concentrations as high as 7,500 ppm. The three major routes for microbial degradation of simazine are dealkylation (removal of the side chains and evolution of carbon dioxide), hydrolysis to form the metabolite hydroxysimazine, and cleavage of the triazine ring (Ghassemi et al. 1981). As one would expect, the highest rates of simazine inactivation are generally found under those conditions that are favorable for microbial growth, such as warm temperatures, high moisture, and high organic matter content (Esser et al. 1975).

Chemical degradation of simazine has been discussed in Section 1.2. Hydrolysis of simazine to form hydroxysimazine is the major route of nonbiological degradation and subsequent loss of activity of simazine in soil, as demonstrated in studies with sterilized soil. Organic matter in the soil, such as humic acid and soil colloids, catalyse this hydrolytic degradation at low pH, while other soil compounds, such as iron and aluminum ions, promote replacement of the chlorine on the triazine ring by the OH^- ion (Khan 1978 in Ghassemi et al. 1981).

Volatilization and photodegradation of simazine are reviewed in Jordan et al. (1970) and Mauck (1974) and are briefly discussed in Section 1.2 of this review. Short wavelengths of light in the ultra-violet range have been postulated to cleave the carbon-chlorine bond of the triazine in laboratory studies. However, the shortest wavelengths of sunlight reaching the earth's surface do not appear to have any significant amounts of photolytic activity with simazine (Pape and Zabik 1969, 1970 in Mauck 1974). Simazine has a relatively low vapor pressure and does not appear to volatilize readily in laboratory studies or in field environments, even at higher temperatures. In forest soils, increased moisture and reduced sunlight should further reduce the small potential for simazine volatilization (Ghassemi et al. 1981).

3.3 Water

The potential fate of simazine in water is of particular concern compared to most other commonly used herbicides because of the use of simazine, especially formulated as Aquazine , for control of aquatic weeds and algae. The fate of simazine in aquatic systems has been reviewed by Ghassemi et al. (1981) and by Mauck (1974).

Simazine is moderately persistent in water, with a reported half-life of 50 to 70 days (U.S. Environmental Protection Agency

registration data cited by Ghassemi et al. 1981). However, this report ignores the dilution and transport effect of flowing rivers and streams. At environmental temperatures and pHs normally found in aquatic systems, chemical degradation (such as hydrolysis) and loss by photolysis or by vaporization are not expected to result in significant rates of simazine dissipation (Ghassemi et al. 1981). Metabolism of simazine by aquatic plants, algae, and bacteria is probably a major route of removal in aquatic systems.

The aesthetic qualities of water containing simazine were examined by Martynyuk and Gzhegotskii (1967 in Mullison 1970). Water containing 0.1 to 5.0 ppm simazine did not differ from control samples in terms of its sensory qualities, even when heated to 140 to 158°F (60 to 70°C) to increase vaporization of noxious substances. Higher concentrations of simazine (50 ppm or greater) resulted in greatly altered sensory (taste and odor) qualities of the water. When bottled ground water containing 600 to 1,000 ppm simazine was stored at 43 to 71.6°F (6 to 22°C) for as long as 20 months, no drop in simazine concentration was observed.

In a study of the effects of simazine treatment of fishponds on resident channel catfish and bluegill populations, Tucker and Boyd (1978) reported that when ponds were treated with simazine at 12 lb/acre (13.4 kg/ha) prior to flooding, simazine concentrations in the water remained above 0.2 ppm for more than 4 months. In addition to a prolonged simazine persistence in these treated ponds, a lower average chlorophyll-a concentration compared to controls existed in the treated ponds throughout the year. However, in this same study, when simazine was added to water at a rate of 1.5 ppm in another group of ponds containing bluegill, the concentrations of simazine decreased rather rapidly to 0.3 ppm within 2 months, for a half-life of 18.5 days.

Several studies report surveys of residues from triazine herbicides, including simazine, in watersheds leading into the Great Lakes as a result of heavy reliance on these herbicides during the middle 1970's. Roberts et al. 1979 studied triazine runoff from an 11,115 acre (4,500 ha) watershed draining into Lake Erie from Ontario, Canada. In this watershed, 0.1 and 0.2 percent of the land was treated with simazine in 1973 and 1974, respectively. Average application rates were 1.28 and 1.67 lb/acre (1.42 to 1.88 kg/ha) respectively in 1973 and 1974. Simazine residues were found in 37 percent of the water samples, with highest concentrations of 3.6 ppb in 1974 when a greater amount of simazine was applied. However, only traces of simazine were found in water at the mouth of the watershed. Only a small fraction of the total amount of simazine applied was ever transported out of the system in water. No simazine residues were detected in three species of fish (bullhead, gizzard shad, and black crappie) sampled. In a second study, Frank (1981)

reported on an analysis of pesticide residues in two Ontario watersheds, one draining into Lake Erie and one draining into Lake Huron. A total application of 1,340 kg of simazine was estimated for 1975 in the two watersheds. Mean unit loadings of simazine for the two watersheds ranged from a low of 0.06 mg/ha/year to a high of 55 mg/ha/year and ranged from a total of 14.35 kg in the 1975-76 year to a total of 39.84 kg in the 1976-77 year. Concentrations of simazine in the discharges of the watersheds into the lakes ranged from below detectable limits to 10 ng/ℓ, with simazine in the water phase of the discharge but not absorbed onto suspended solids. In general, agricultural applications of simazine appear to result in small concentrations of residues (if any) in stream water, and very small amounts being transported out of the watershed by stream flow.

In a study of simazine persistence in small (0.25 acre) ponds, Mauck et al. (1976 in Ghassemi et al. 1981) treated ponds so as to achieve treatment concentrations of 0.1 to 3.0 ppm. After 346 days, he found simazine present at concentrations ranging from 0.01 to 0.14 ppm. When the treatment for these ponds was repeated 1 year after initial application, the concentration of simazine was much less, with 0.01 to 0.50 ppm residues at 456 days. Simazine residues in water in this study were directly proportional to the rates of application. Actual amounts of residues were generally no more than one-third to one-half of the total herbicide applied. The slower rate of simazine disappearance from these ponds following the second application may be due to lower populations of algae (observed in other studies such as Tucker and Boyd 1978 and Sutton 1965 in Mauck 1974) which metabolize simazine (Fankhauser 1967 in Mauck 1974).

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to simazine during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved simazine. Calculations of exposure to simazine in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements, with the exception that only ground application is considered in the analysis. In general, it should be noted that environmental exposures to simazine are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, will be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Simazine Application

Available application data for simazine use by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to simazine differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of simazine in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method of application, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Simazine is readily adsorbed to soil particles. It has relatively low solubility in water and has moderate mobility in soil. Simazine is moderately persistent in soil and has been detected in the top portions of the soil up to four years after application. Chemical and microbial degradation are the primary mechanisms of simazine dissipation from soil.

4.2 Exposure

Estimates of potential exposure to simazine are based on ground application since the Forest Service did not employ aerial application methods in 1982 or 1983. The compound was applied from the ground (ground foliar) at rates as high as 12.0 lb a.i./acre. Those

Table 4-1

USDA Forest Service Application Data for Simazine (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Simazine (18)	Ground Foliar and Granular	2.2	40	4.9	3.1	6.1	4.6	Research (5) Timber (4) Noxious Weeds (3) Engineering (3) Nursery (2) Wildlife (1)

Source: Gross 1983

calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose and wildlife oral exposures), assume an application rate of 12.0 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of simazine and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses

Data are not available on the daily occupational exposures or doses of simazine to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb a.i./acre. Daily occupational dose estimates for simazine are based on exposures on a per pound per acre application rate multiplied by 12.0 lb/acre for backpack sprayers.

For ground applications at the 12 lbs a.i./acre application rate, the following estimate of daily dose is derived:

Backpack Sprayer: 0.48 mg/kg (0.04 mg/kg at 1 lb/acre application rate)

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg. No dose is calculated for observers, since simazine is used only in ground applications.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by simazine application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide.

Table 4-2

Summary of Occupational Dose, Environmental Exposure and Toxicity for Simazine

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg		[b]
Observer	No dose calculated. See text.		
Backpack Sprayer	0.48 mg/kg		
Environmental Exposure			
Fish	Negligible residues. See text.		
Rabbits	2.42 mg/kg	Negligible	12.6 mg/kg
Deer	0.61 mg/kg	Negligible	17.3 mg/kg
Man	--[c]	--[c]	--[c]
(Fish)	NA[d]	NA	0.0 mg/kg
(Rabbits)	NA	NA	0.055 mg/kg (2 days)
(Deer)	NA	NA	0.070 mg/kg (2 days)
(Water)	NA	NA	0.0 mg/kg
(Berries)	NA	NA	0.0 mg/kg
(Mushrooms)	NA	NA	0.0 mg/kg
Toxicity Summary	LD_{50} >10,000 mg/kg in rabbits.	1 hour dust exposure to 1.8 to 4.9 ppm in rats caused no toxic effects.	Acute oral LD_{50}'s >5,000 mg/kg in rats, mice, and rabbits. Chronic feeding of 100 ppm for 2 years in rats caused by no observed effects (NOEL).
	Most studies, LC_{50} >10 ppm for fish		

[a] It was assumed, based on 1982 and 1983 Forest Service data, that simazine is not applied aerially on Forest Service sites.

[b] Occupational doses include all routes of exposure.

[c] Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[d] NA represents nonapplicable data.

Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive an exposure much less (by orders of magnitude) than that of the mixer/loader or backpack sprayer.

It was assumed that animals would not be sprayed directly by backpack applicators, and that only the animals' ventral surfaces would contact foliage that had been sprayed. Thus, approximately one-half of the animals' body surface would contact sprayed vegetation, resulting in an exposure level equal to one-half of that calculated for whole-body exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on backpack applications of 12.0 lb a.i./acre.

Rabbits in Aerial Spray Zone: 2.418 mg/kg (1/2 x 0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.606 mg/kg (1/2 x 0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure much less (by orders of magnitude) than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Simazine has a very low vapor pressure at ambient temperatures, and it is unlikely that humans and other animals will be exposed to more than negligible amounts of vapor. Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 50 feet between ground foliar spray zones and waterbodies, environmental exposures for aquatic organisms would be limited to the amounts of simazine carried into the stream or pond in runoff water. Simazine has a very low solubility in water, adheres strongly to soils, is not readily leached, and is not subject to

drift, since it is not applied by aerial means. Therefore, the amounts of simazine that could be expected to occur in aquatic systems as a result of USDA Forest Service applications are assumed to be negligible and, for the purpose of analysis here, water concentrations are assumed to be zero.

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of simazine by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed at 12.0 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 17.28 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 12.60 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of simazine in fish, deer, and rabbit.

Fish tissue concentrations are assumed to be zero since negligible amounts of simazine are expected to leach into waterbodies from ground applications. Deer and rabbit tissue concentrations include simazine accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied simazine is absorbed at a rate of 10 percent. The assumption of 10 percent dermal absorption of simazine is conservative, since the low solubility of simazine in water and organic solvents indicates little potential for dermal absorption. In addition, metabolism studies summarized in Appendix F indicate that only small amounts of ingested simazine are absorbed by the gut and only small amounts of tissue residues are found in animals exposed to simazine. It is also assumed that oral exposure of deer and rabbit to simazine lasts only for 2 days due to the relatively short environmental persistence of simazine. Deer tissue concentrations of simazine are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.606 mg/kg (dermal exposure) x 0.1 = 0.0606 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 17.28 mg/kg/day (oral exposure) x 0.10 x 2 days = 3.456 mg/kg.

Total Deer Tissue concentration = 0.0606 mg/kg + 3.456 mg/kg = 3.5166 mg/kg (3.517 ppm).

Rabbit tissue concentrations of simazine are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 2.418 mg/kg (dermal exposure) x 0.1 = 0.2418 mg/kg.

Oral Exposure contribution assumes 10 percent absorption of two-days feeding, or 12.60 mg/kg/day (oral exposure) x 0.10 x 2 days = 2.520 mg/kg.

Total Rabbit Tissue Concentration = 0.2418 mg/kg + 2.520 mg/kg = 2.762 mg/kg (2.762 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to simazine from eating meat are derived:

Fish meat: = 0 mg/kg

Deer meat: 3.517 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.070 mg/kg

Rabbit meat: 2.762 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.055 mg/kg

Water and plant consumption by man would result in no oral exposures to simazine if there is no aerial application. Simazine residues in waterbodies are assumed to be negligible. Ground appliction of simazine is assumed to result in negligible residues, if any, on berries and mushrooms outside of the posted limits of the 50-foot spray buffer zone.

4.3 Hazard Assessment

Toxicological properties of simazine to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rat inhalation results, rabbit and rodent acute oral LD_{50}'s, rat

chronic oral NOEL, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels anticipated for USDA Forest Service applications. Based upon chronic feeding studies with rats, simazine concentrations of 100 ppm in the diet caused no observable effect. Assuming a 100-fold margin of safety for extrapolation to man (a factor of 10 for inter-species variability and a factor of 10 for human intra-species variability), one can assume that 100 mg/kg/day ÷ 100, or a 1 mg/kg/day (1 ppm) oral dose in man, will not result in adverse effects. The estimated human exposures from ingestion of game are well below 1 mg/kg per day.

Small mammal studies have shown that simazine applied to the eye causes only transient inflammation and applied to skin or by inhalation causes no adverse toxic effects. There is no evidence that simazine is teratogenic. Simazine was negative in most mutagenicity test systems. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive or, mutagenic effects to individuals from simazine use, if proper care is taken during application. Based on lifetime studies in mice, there should be no significant potential for adverse carcinogenic effects to individuals from simazine use.

Appendix A

Summary of Simazine Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine (Technical material, 98.1% a.i.)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 1.1 ppm (0.56 to 2.2)[a]	21°C, first instar	Johnson and Finley 1980
Simazine (Technical material, 98.1% a.i.)	Ostracod (Cypridopsis sp. ; Crustacea)	Surface absorption, static bioassay	48 hours	EC_{50} = 3.7 ppm (2.6 to 5.3)	21°C, mature (original report by Sanders [1970] report TL_{50} = 3.2 ppm).	
Simazine (Technical material, 98.1% a.i.)	Amphipod (Gammarus fasciatus; Crustacea)	Surface absorption, static bioassay	96 hours	LC_{50} 100 ppm	15°C, mature	
Aquazine® (80% Simazine a.i.)	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption, static bioassay	48 hours	LC_{50} = 5.3 ppm (2.2 to 8.4) LC_{20} = 21.3 ppm (14.8 to 31.9) LC_{50} = 92.1 ppm (54.3 to 259.1)	24 ± 12-hour-old individuals were used. Temperature was 20 ± 1°C, pH 7.4 to 7.7, total alkalinity 100 to 116 ppm.	Fitzmayer et al. 1982
Aquazine® (80% Simazine a.i.)	Cladoceran (Daphnia pulex; Crustacea)	Surface absorption, static bioassay with daily replacement of test solutions	up to 26 days	Survival in 20 ppm averaged 9.6 ± 1.7 days, at 4 ppm, 65% mortality after 25 days.	At 4 ppm and 20 ppm, simazine interfered with molting and caused reduced growth. At 4 ppm, reproductive maturation was delayed and numbers of live young and broods were lower than controls. At 20 ppm, reproductive maturation was prevented.	Fitzmayer et al. 1982
Simazine[b]	Amphipod (Gammarus lacustris; Crustacea)	—[c]	48 hours	LC_{50} = 21 ppm	--	U.S. Department of the Interior 1968 in Pimentel 1971

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine	Amphipod (Gammarus lacustis; Crustacea)	—	24 hours	LC_{50} = 30 ppm	--	Sanders 1969 in Pimentel 1971
		—	96 hours	LC_{50} = 13 ppm	--	Saunders 1969 in Hurlbert 1975
Simazine (Technical)	Sowbug (Asellus brevicaudus; Isopoda)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	15.5°C, pH 7.4, total alkalinity 260 ppm.	Sanders 1970
Simazine (Technical)	Glass shrimp (Palaemonetes kadiakensis; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	21°C, pH 7.4, total alkalinity 260 ppm.	
Simazine (Technical)	Crayfish (Orconectes nais; Crustacea)	Surface absorption, static bioassay	48 hours	TL_{50} >100 ppm	24°C, pH 7.4, total alkalinity 260 ppm.	
Simazine	Pink Shrimp (Crustacea)	—	96 hours	NOEL at 75 ppm	--	U.S. Environmental Protection Agency registration data reported by Ghassemi et al. 1981
Simazine	Mud Crab (Crustacea)	—	96 hours	NOEL at 1,000 ppm	--	
Simazine	Cladoceran (Daphnia sp.; Crustacea)	Surface absorption, flow-through bioassay	—	No effects on daphnid reproduction were observed at concentrations of 0.25 to 3.0 ppm.	--	Mayer and Sanders 1977 in Ghassemi et al. 1981

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Tafazine® (Simazine)	Freshwater Zooplankter-copepod (Heliodiaptomus viduus; Crustaea)	Surface absorption	up to 51 hours	At 1.0 ppm, 50% mortality in 24 hours and 100% mortality 51 hours. At 10.0 ppm, 50% mortality in 13 hours and 100% mortality in 34 hours. At 50.0 ppm, 50% mortality in 11 hours and 100% mortality in 16 hours. At 100.0 ppm, 50% mortality in 5 hours 100% mortality in 12 hours.	--	George et al. 1982
Simazine (Technical material, 98.1% a.i.)	Plecopteran-Stonefly (Pteronarcys sp.; Insecta)	Surface absorption, static bioassay	96 hours	LC_{50} = 1.9 ppm (0.9 to 4.0)	15°C, 2nd year class	Johnson and Finley 1980
Simazine	Plecopteran-Stonefly (Pteronarcys californica; Insecta)	--	48 hours	LC_{50} = 50 ppm	—	U.S. Department of the Interior 1968 in Pimentel 1971
Simazine	Midges (Insecta)	Surface absorption, flow-through bioassay	—	Midge emergence was temporarily delayed at concentrations of 0.66 and 2.2 ppm.	—	Mayer and Sanders 1977 in Ghassemi et al. 1981
Simazine	Soil Arthropods (not specified)	Field exposure	Several years	1.5 lb/acre (1.68 kg/ha) applied twice per year resulted in significantly reduced numbers of soil arthropods compared to controls (hand weeded plots).	Simazine applied 2-times each year to two plots, two control plots weeded by hand. Soil arthropods sampled each month.	Edwards and Stafford 1979

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Tafazine® (50% a.i.)	Midge (Chironomus tentans; Insecta, larvae)	Surface absorption, static bioassay	48 hours	LC_{50} = 3.58 ppm	LC_{50} value as active ingredient. pH 8.3, 24.5 to 25.2°C	Dad and Tripathi 1980
Simazine	Oyster (Mollusca)	--	--	No inhibition of shell growth at 1.0 ppm.	--	U.S. Environmental Protection Agency registration data reported by Ghassemi et al. 1981
Simazine	Snails (Goniobasis livescens and Viviparus georgianus; Gastropod molluscs)	Field exposure and laboratory test	96-hour laboratory test	At 0.5 ppm in lake, popula- (biomass) decline observed; in laboratory, 5.0 ppm tolerated up to 96 hours.	High mortality in field study may be due to synergism between herbicide effects and death of algae.	Harmon 1978 in Buikema 1981
Simazine	Earthworm (Lumbricus sp.; Annelida)	Surface absorption, chronic	32 days	1 of 8 worms killed at doses equivalent to both 3 and 12 lb/acre (3.4 and 13.4 kg/ha).	Worms exposed in potted soil.	DeVries 1962 in Pimentel 1971
Simazine	(Helodrilus sp.)	Surface absorption, chronic	32 days	No deaths observed in 32 individuals at doses equivalent to both 3 and 12 lb/acre (3.4 and 13.4 kg/ha).	Exposure in potted soil.	
Tafazine® (50% a.i.)	Freshwater Ectoproct (Plumatella casmiana)	Surface absorption	--	Concentrations of 0.01 and 1.5 ppm resulted in reduction in rate of statoblast germination.	--	Rao and Dad 1979

Appendix A (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine	Soil worms (nematodes enchytraea, annelids) and mites	Field exposure in "Forest Garden" and grass covered pine plantation.	--	High doses of 4.5 and 6.7 lb/acre (5.0 and 7.5 kg/ha inhibited populations of soil soil organisms in the "forest garden" but resulted in population growth in the pine planation.	--	Ghassemi et al. 1981 (studies by Baimber et al. or Baumber et al. 1978 quoted in Ghassemi et al. 1981)
Simazine	Mayflies, mosquitoes, biting midges, damselfly nymphs, water beetles, aquatic worms, leeches, and snails	--	--	Population reduction of 50% or more a after application ranging from 0.5 to 10.0 ppm.	--	Walker 1962 in Pimentel 1971
Simazine	Common midges and aquatic worms	Surface absorption	96 hours	Approximately 50% killed at at a dosage of about 28 ppm.	70°F, slightly alkaline pH, 21 to 24 ppm hardness.	Walker 1964 in Pimentel 1971 and in Mauk 1974
Simazine	Fungus (Aspergillus fumigatus)	Simazine in culture medium	--	At 5 ppm simazine, fungus was able to metabolize simazine as a source of carbon.	--	Kaufman et al. 1965
Simazine	Soil fungus	--	--	Fungal populations slightly stimulated at simazine concentrations of 2.5, 5.0, 10.0 ppm.	Sandy loam soil.	Navyar et al. 1970 in Ghassemi et al. 1981

Appendix A (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine	Soil microorganisms	--	--	Enzyme activity (urease) stimulated at a dose equivalent to 1.8 lb/acre (2 kg/ha) and inhibited at 8.9 lb/acre (10 kg/ha).	--	Manorik and Malichenko 1969 in Grossbard and Davies 1976
Simazine	Soil microorganisms	--	--	Nitrification inhibited at simazine concentration of 6 ppm.	--	Quoted by Audus 1970 in Grossbard and Davies 1976
Simazine	Soil microorganisms	Simazine incorporated into potted soil in greenhouse	7 days	At dose equivalent to 3.6 lb/acre (4 kg/ha) fungi and azotobacter populations were stimulated and bacteria and actinomycetes populations were unaffected.	In red sandy soil.	Bopaiah and Rai 1979
Simazine	Soil microorganisms	Simazine applied at "normal application rates" for weed control.	--	Did not significantly affect the relative numbers of fungi and bacteria or growth of some fungi.	--	Eno 1962 in Pimentel 1971
Simazine	Soil microorganisms	--	--	Simazine at "normal and above normal field application rates" did not affect nitrogen cycling microorganisms but did stimulate cellulolytic microorganisms.	--	Torstenssen 1974 in Ghassemi et al. 1981

[a]95 percent confidence limits in parenthesis.
[b]No information provided on actual amounts of active ingredient.
[c]Data are not available or not applicable.

Appendix B

Summary of Simazine Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine	Striped bass	Surface absorption	24 hours	LC_{50} = 0.6 ppm	--[a]	Wellborn 1969 in Pimentel 1971
Simazine	Rainbow trout	—	24 hours	LC_{50} = 68 ppm	--	Cope 1965 in Pimentel 1971
Simazine	Bluegill	—	24 hours 48 hours	LC_{50} = 130 ppm LC_{50} >100 ppm	--	Cope 1965 in Pimentel 1971 and in Sanders 1970
Simazine	Major carp	Surface absorption, static bioassay	96 hours	50 ppm - 100% mortality in 2 hours. 25 ppm - 40% mortality in 96 hours. 1 ppm - 9% mortality in 96 hours.	Sensitivity to herbicide increased in presence of weeds- LD_{50} = 7.5 ppm.	Singh and Yadav 1978
Simazine	Rainbow trout	—	48 hours	LC_{50} = 5 ppm	--	U.S. Department of the Interior 1968 in Pimental 1971
Simazine	Rainbow trout	—	48 hours	LC_{50} = 56 ppm	--	Bohmont 1967 in Pimentel 1971
	Bluegill	—	48 hours	LC_{50} = 118 ppm	--	
Simazine	Rainbow trout	Surface absorption	96 hours	LC_{50} = 5.6 ppm	--	Cope 1965 in Mauck 1974
Simazine	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 2.8 ppm	61°F, 30 ppm hardness	Test data for Ciba-Geigy Corporation from Woodard Research Corporation 1965 cited in Mauck 1974

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine	Goldfish	Surface absorption, static bioassay	96 hours	LC_{50} 32 ppm	57°F, 30 ppm hardness	Test data for Ciba-Geigy Corporation from Woodard Research Corporation 1965 cited in Mauck 1974
Simazine	Bluegill static bioassay	Surface absorption,	96 hours	LC_{50} = 16 ppm hardness	64°F, 30 ppm	
Simazine	Bluegill	Surface absorption	96 hours	LC_{50} = 11.6 ppm	--	Cope 1965 in Mauck 1974
Simazine	Mirror carp (sic.)	Surface absorption	--	First toxic signs noted after 8 hours at 1,000 ppm.	--	U.S. Environmental Protection Agency registration data cited by Ghassemi et al. 1981
Simazine	Rainbow trout	Surface absorption	--	Toxic signs after 4 to 8 hours at 100 ppm .	--	
Simazine	Bluegill	Surface absorption, field ponds	7 weeks	No noticeable effect at 7 ppm concentration in small test ponds.	--	
Simazine	Fathead minnow	Surface absorption	96 hours	NOEL at 2.5 ppm	--	
Simazine (80W)	Carp x Goldfish (hybrid)	Surface absorption, static bioassay	96 hours	LC_{50} = 190.0 ppm	20°C, 21 to 23 ppm hardness	Walker 1964 in Mauck 1974
Simazine (80W)	Bluntnose minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 63 ppm		
Simazine (80W)	Channel catfish	Surface absorption, static bioassay	96 hours	LC_{50} = 85 ppm		
Simazine (80W)	Pumpkinseed	Surface absorption, static bioassay	96 hours	LC_{50} = 28 ppm		
Simazine (80W)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 36 ppm		

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine (80W)	Redear sunfish	Surface absorption, static bioassay	96 hours	LC_{50} = 55 ppm	20°C, 21 to 23 ppm hardness	Walker 1964 in Mauck 1974
Simazine (80W)	Largemouth bass	Surface absorption, static bioassay	96 hours	LC_{50} = 45 ppm		
Simzaine (80W)	Yellow perch	Surface absorption, static bioassay	96 hours	LC_{50} = 100 ppm		
Simazine (80W)	Channel catfish	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	pH 7.9	Jones 1962 in Mauck 1974
Simazine (80W)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm		
Simazine (80W)	Largemouth bass	Surface absorption, static bioassay	96 hours	LC_{50} = 25.0 ppm		
Simazine (Granular)	Bluegill		12 days	10 ppm = highest concentration with no mortality for 12 days.	Small fish (1 to 3 inch).	Hiltibran 1967
Simazine (Granular)	Bluegill	Surface absorption, static bioassay	8 days	No morality at 10 ppm for 8 days.	Fry.	Hiltibran 1967
	Green sunfish		7 days	Survived 7 days at at 10 ppm.		
	Lake chubsucker		8 days	No mortality at 10 ppm for 8 days.		
	Smallmouth bass		8 days	No mortality at 10 ppm for 8 days.		
Simazine (Dissolved in 0.01 to 0.03% Acetone)	Rainbow Trout	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} >100 ppm LC_{50} >100 ppm	14 ± 2°C, pH 6.8 to 7.6.	Bathe et al. 1975
	Crucian carp		48 hours 96 hours	LC_{50} >100 ppm LC_{50} >100 ppm	14 ± 2°C, pH. 6.8 to 7.6.	
	Catfish		48 hours 96 hours	LC_{50} = 80 ppm LC_{50} = 65 ppm	20 ± 2°C, pH 6.8 to 7.6.	

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine (Dissolved in 0.01 to 0.03% Acetone)	Perch	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} >100 ppm LC_{50} = 90 ppm	14 + 2°C, pH 6.8 to 7.6.	Bathe et al. 1975
	Guppy	Surface absorption, static bioassay	48 hours 96 hours	LC_{50} >49 ppm LC_{50} = 49 ppm	20 + 2°C, pH 6.8 to 7.6.	
Simazine (Dissolved in 0.1% Acetone)	Rainbow trout	Surface absorption, chronic toxicity, static bioassay	56 days	2.5 ppm for 28 days. No acute or delayed toxic symptons observed. No mortality observed.	Fish were kept in water with simazine for 28 days, then placed in herbicide-free water for 28 days (total 56 days). No treatment-related macroscopic or microscopic effects were observed.	Bathe et al. 1975
Simazine (Technical material, 98.1% a.i.)	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	12°C, 1.2g fingerlings.	Johnson and Finley 1980
Simazine (Technical material, 98.1% a.i.)	Fathead minnow	Surface absorption, static bioassay	96 hours	LC_{50} >100 ppm	25°C, 0.7g fingerlings.	
Simazine (wettable powder)	Rainbow trout	Surface absorption	24 hours	LC_{50} = 95 ppm	--	Alabaster 1969 in Dodson and Mayfield 1979
Simazine (wettable powder)	Bluegill	Surface absorption, static bioassay	12 days	30 ppm = highest concentration with no mortality for 12 days	Small Fish (1 to 3 inch).	
Simazine (wettable powder)	Green sunfish	Surface absorption, static bioassay	5 days	Survived 5 days at 10 ppm.	Fry.	Hiltibran 1967
	Lake chubsucker		8 days	No mortality at 10 ppm for 8 days.		
	Smallmouth bass		3 days	Survived 3 days at 3 ppm.		

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine (80% wettable powder)	Striped bass	Surface absorption, static bioassay	96 hours	LC_{50} = 0.25 ppm	McCann and Hitch (1980) were unable to duplicate these results and hypothesized that the presence of an additive and/or differences in handling of the fish may have resulted in different results. Fingerlings.	Wellborn 1969 in McCann and Hitch 1980
Simazine (80% wettable powder)	Striped bass	Surface absorption, static bioassay and field exposures	up to 21 days	No treatment-related mortality in aquarium fish exposed to 3 ppm or in pond fish with 3 ppm applied once a week for three weeks.	Fingerlings.	Cook and Smith 1976 in McCann and Hitch 1980
Simazine (80% a.i. wettable powder)	Bluegill	Surface absorption, static bioassay	96 hours	LC_{50} = 100 ppm (90 to 110 ppm)[b]	24°C, 1.0g Fingerlings.	Johnson and Finley 1980
Simazine (Technical) + Tween 80® (Wetting agent)	Rainbow trout	Surface absorption	24 hours	Exposure to 1.0, 4.0, 12.5 ppm simazine with wetting agent resulted in decreased response to currents and a decrease in swimming speed compared to controls after 24-hour exposure.	Behavioral effects assumed to be due to the Tween 80 wetting agent that was added only to the technical grade simazine. Yearling fish.	Dodson and Mayfield 1979
Princep® 80W	Rainbow trout	Surface absorption static bioassay	24 hours	No mortality observed at 200 ppm a.i.	Yearling fish.	Dodson and Mayfield 1979

Appendix B (Continued)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Princep® 80W	Bluegill and Channel catfish	Surface absorption	96 hours	LC_{50} >1,000 ppm for both species.	--	U.S. Environmental Protection Agency registration data cited by Ghassemi et al. 1981
Aquazine® (80% wettable powder)	Striped bass	Surface absorption, static bioassay	96 hours	LC_{50} >180 ppm	Fish were tested in both hard and soft water at 21 ± 1°C, pH 6.9. Fingerling.	McCann and Hitch 1980
Aquazine® (80% a.i.)	Channel catfish	Surface absorption	up to 6 months	Nineteen percent less yield from ponds in which bottoms had been treated with 12 lbs. (a.i.)/acre (13.4 kg/ha) one day prior to filling.	Concentration of simazine in pond water remained above 0.2 ppm for more than 4 months. Dissolved oxygen levels in experimental ponds remained lower than controls throughout the 6 months.	Tucker and Boyd 1978
Aquazine® (80% a.i.)	Bluegill	Surface absorption	up to 128 days	Single application of herbicide at 1.5 ppm to fertilized bluegill ponds resulted in an 11% reduction in fish yield.	Concentration of simazine in pond water decreased from 1.5 ppm to 0.3 ppm within 2 months with a half-life of 18.5 days. Dissolved oxygen concentrations decreased rapidly following application of simazine but in 2 months were at levels comparable with controls.	Tucker and Boyd 1978

Appendix B (Concluded)

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Tafazine®	(*Sarotherodon mossambicus*) Indian teleost	Surface absorption, static bioassay	96 hours	LC_{50} = 3.1 ppm LC_{100} = 8.0 ppm	Water temperature was 28.0 to 29.0°C, pH 8 to 9. Rate of operular beating was observed to increase with increasing toxic concentration.	Rao and Dad 1979
Tafazine®	(*Puntius ticto*) Indian teleost	Surface absorption, static bioassay	96 hours	LC_{50} = 24.5 ppm LC_{100} = 64.0 ppm		
Tafazine®	(*Danio* sp.) Indian teleost	Surface absorption, static bioassay	96 hours	LC_{50} = 12.6 ppm LC_{100} = 40.0 ppm		
Tafazine® (50% a.i.)	Major Carp	Surface absorption, static bioassay	24 hours 48 hours 96 hours	LC_{50} = 8.6 ppm LC_{50} = 5.0 ppm LC_{50} = 2.5 ppm	Median lethal concentrations expressed in parts a.i. Water temperature was 24.5 to 25.2°C, pH 8.3, hardness 230 ppm. General responses of fish to toxic stress included loss of equilibrium, increased mucus secretion, increased rate of excretion, and, at higher toxic concentration, loss of pigment.	Dad and Tripathi 1980
Tafazine® (50% a.i.)	(*Mystus vittatus*) Indian teleost	Surface absorption, static bioassay	24 hours 48 hours 96 hours	LC_{50} = 59.6 ppm LC_{50} = 40.0 ppm LC_{50} = 28.6 ppm		
Tafazine® (50% a.i.)	(*Labeo rohita*) Indian teleost	Surface absorption, static bioassay	24 hours 48 hours 96 hours	LC_{50} = 12.2 ppm LC_{50} = 5.4 ppm LC_{50} = 2.5 ppm		

[a]Data not available or not applicable.
[b]95 percent confidence limits in parentheses.

Appendix C

Summary of Simazine Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Simazine (99.1%)	Bobwhite quail	Ingestion of stated dose in feed in ppm	Fed dose for 5 days, observed 3 days after cessation.	LC_{50} >5,000 ppm	No mortality observed up to stated dose. Birds were 10 to 12 days old at initiation of test.	Hill et al. 1975
	Japanese quail			LC_{50} >3,720 ppm		
	Ring-necked pheasant			LC_{50} >5,000 ppm		
	Mallard			LC_{50} >5,000 ppm		
Simazine (80W)	Bobwhite quail	Ingestion	5 to 7 days	LC_{50} >8,800 ppm	Review did not explicitly state the nature of the reported dose. In context it is assumed to be an LC_{50}.	Ciba-Geigy Chemical Corporation 1970 in Mauck 1974
	Mallard	Ingestion	5 to 7 days	LC_{50} >51,200 ppm		
Simazine (80% wettable powder)	Chicken	Ingestion	Acute	LD_{50} >5,000 mg/kg	No mortality observed.	Palmer and Radeleff 1969
	Pigeons	Ingestion	Acute	LD_{50} >5,000 mg/kg	No mortality observed.	
	Chickens	Ingestion by capsule	10 days	Chickens dosed at 50 mg/kg body weight or more showed a reduced weight gain compared to controls.	One chicken at autopsy had an enlarged and congested liver as well as a congested intestinal lining.	
Simazine (HCl)	Chicken (eggs)	Injection (75% acetone solvent)	—[a]	Decreased proportion of hatching compared to controls at doses giving a concentration of 300 ppm (94%). No effect at 200, 100, or 10 ppm.	--	Dunachie and Fletcher 1970
Simazine	Mallard	Ingestion	—	Dietary levels of 2.0 and 20.0 ppm before and during normal egg production cycles did not affect reproductive function.	--	Fink 1975 in Ghassemi et al. 1981

[a]Data not available or not applicable.

Appendix D

Summary of Simazine Toxicity Data for Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Simazine	Rat	Ingestion of a single dose	LD_{50} >5,000 mg/kg	—[a]	U.S. Department of Agriculture 1981
	Mouse	Ingestion of a single dose	LD_{50} >5,000 mg/kg	—	
	Rabbit	Ingestion of a single dose	LD_{50} >5,000 mg/kg	—	
Simazine	Prairie vole (Microtus ochrogaster; male)	Single dose by gavage	LD_{50} (95% confidence limits) = 3,920 (3,440 to 4,570) mg/kg	—	Cholakis et al. 1981
	Prairie vole (Microtus ochrogaster; female)	Single dose by gavage	LD_{50} (95% confidence limits) = 3,250 (2,640 to 3,980) mg/kg	—	
	Gray-tailed vole; (Microtus canicaudus male)	Single dose by gavage	LD_{50} (95% confidence limits) = 2,010 (1,400 to 2,900) mg/kg	—	Cholakis et al. 1981
	Gray-tailed vole; (Microtus canicaudus; female)	Single dose by gavage	LD_{50} (95% confidence limits) = 2,360 (2,090 to 2,690) mg/kg	—	
			Acute Dermal Toxicity		
Simazine	Rabbit	One dose applied to skin	LD_{50} = >10,000 mg/kg	—	U.S. Department of Arigculture 1981

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity		
Simazine (80% wettable powder)	Cattle	10 mg (a.i.)/kg/day, 10 doses by drench	No ill effects observed.	Poisoning was considered to have occurred when any sign of abnormal function or behavior was observed. These ranged from loss of appetite, depression, muscular spasms, dyspnea, weakness, uncoordinated gait, and moribundity. A weight loss of 5% or more was considered significant.	Palmer and Radeleff 1969
	Cattle	20 mg/kg/day, 6 doses by drench	Poisoned after 3 doses, survived with 5% weight.		
	Cattle	25 mg/kg day, 10 doses by drench	Poisoned survived with 12% weight loss.		
	Cattle	50 mg/kg/day, 10 doses by drench	Poisoned after 3 doses, survived, 21% weight, loss, long recuperative period.		
	Cattle	100 mg/kg/day, 7 doses by drench	Poisoned after 3 doses, sacrificed after 7 doses when moribund. Necropsy showed congestion of lungs and kidneys; swollen, friable liver; petechiae on surface of the pericardium.	Poisoning was considered to have occurred when any sign of abnormal function or behavior was observed. These ranged from loss of appetite, depression, muscular spasms, dyspnea, weakness, uncoordinated gait, and moribundity. A weight loss of 5% or more was considered significant.	Palmer and Radeleff 1969
	Cattle	250 mg/kg/day, 3 doses by drench	Poisoned after 1 dose, survived with 11% weight loss.		
	Sheep	25 mg (a.i.)/kg/day, 10 doses by drench	No ill effects observed.	Criteria for poisoning were the same as for cattle. Necropsy findings were the same as for cattle.	Palmer and Radeleff 1969
	Sheep	50 mg/kg/day, 31 doses by drench	Poisoned after 17 doses; died 14 days later with 13% weight loss.		
	Sheep	50 mg/kg/day, 10 doses by drench	Poisoned and survived with 18% weight loss.	Criteria for poisoning were the same as for cattle. Necropsy findings were the same as for cattle.	Palmer and Radeleff 1969
	Sheep	100 mg/kg/day, 14 doses by drench	Poisoned after 4 doses and died after 14 doses.		
	Sheep	100 mg/kg/day, 10 doses by capsule	Poisoned, with 13% weight loss; died 4 days after last dose.		

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Concluded)		
Simazine	Sheep	250 mg/kg/day, 3 doses by drench	Poisoned after 1 dose; survived with 9% weight loss.	Criteria for poisoning were the same as for cattle. Necropsy findings were the same as for cattle.	Palmer and Radeleff 1969
	Sheep	250 mg/kg/day, 10 doses by capsule	Poisoned after 3 days and died.		
	Sheep	400 mg/kg/day, 9 doses by drench	Poisoned after 3 days and died.		
			Dermal Irritation		
Simazine	Rabbit	21-day repeated application	LD_{50} = 2,000 mg/kg. No substantial skin irritation when 200 mg/kg were applied.	--	U.S. Department of Agriculture 1981
			Eye Irritation		
Simazine	Unspecified	Unspecified	No serious eye irritation has been reported from experimental or commercial use.	--	U.S. Department of Agriculture 1981
Simazine	Rabbit	71 mg/kg applied to eye	No irritation to iris or cornea, transient inflammation of conjunctivae.	(Not in Herbicide Handbook, Weed Science Society of America 1979 which was cited in U.S. Department of Agriculture 1981).	
			Inhalation Toxicity		
Simazine 80W (80% wettable powder)	Rat	One hour exposure to dust aerosol concentration ranging from 1.8 to 4.9 mg/m^3	No deaths or signs of toxicological or pharmaceutical effects.	--	Weed Science Society of America 1983

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Chronic Toxicity		
Simazine 50W (50% wettable powder)	Rat	2-year feeding study with concentrations as high as 100 ppm in the diet	No gross or microscopic signs of systemic toxicity.	—	U.S. Department of Agriculture 1981; Weed Science Society of America 1983
			Reproductive and Teratogenic		
Simazine	Rat	Daily inhalation for 1 to 3 hours on days 7 through 14 of gestation. Animals exposed to concentrations of 17, 77 and 317 mg/m^3	No treatment related teratogenic effects were observed.	—	Newell and Dilley 1978
			Carcinogenicity		
Simazine	Mouse	Given maximum tolerated doses of 215 mg/kg from days 7-28; 603 ppm in diet for approximately 18 months	No treatment related tumor induction.	—	Innes et al. 1969
Fogard S® (37.5% simazine and 25% atrazine)	Mouse	13 subcutaneous injections of 0.25 ml of Fogard S® (2 ppm) at 3-day intervals for total of 0.0065 mg active ingredient	3 of 24 (12.5%) animals died with malignant lymphomas. One animal (4.2%) had peritoneal mesothelioma.	Animals observed for 7 months. No tumors in controls.	Donna et al. 1981

[a]Data not available or not applicable.

Appendix E

Summary of Simazine Mutagenicity Data

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Simazine	Salmonella typhimurium	Histidene-positive revertant mutant assay. Strains TA100, TA1535, TA1537, and TA1538 treated with concentrations of 1 to 1,000 g per plate in the absence or presence of rat liver S-9 homogenate for metabolic activation.	Nonmutagenic. No increase in the number of histidine-independent revertants was observed.	--[a]	Simmon 1978
Princep®	S. typhimurium	Strains TA1535, TA1537, TA1538, TA98, and TA100 were treated with up to undiluted material (10 μℓ per plate) in the absence or presence of rat liver S-9 homogenate.	Nonmutagenic. No increase in revertant mutants.	--	Eisenbeis et al. 1981
Princep® + Atrazine®	S. typhimurium	Strains TA1535, TA1537, TA1538, TA98, and TA100 were treated with up to undiluted solution of the muture (10 μℓ per plate) in the absence or presence of rat liver S-9 homogenate.	Nonmutagenic. No increase in revertant mutants.	--	Eisenbeis et al. 1981
Simazine	S. typhimurium	Strain TA1537 treated with 1, 10, or 100μℓ/plate in the presence of S-9 homogenate prepared from rat liver, kidney, brain, spleen, lung, stomach, or blood.	Nonmutagenic. No increase in revertant mutants.	--	Commoner 1976

Appendix E (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Simazine	Escherichia coli WP2 (uvrA⁻)	Assay measures reversion of WP2 tryptophan independence. Strain WP2 treated with concentrations of 1 to 1,000 μg per plate in the absence or presence of rat liver S-9 homogenate.	Nonmutagenic. No increase in the number of tryptophan-independent revertants was observed.	--	Simmon 1978
Simazine	E. coli	Assay measures preferential killing of DNA repair-deficient strain (p 3478) over wild type strain (w 3110). Cells treated with 1.0 mg per disc per plate.	Nonmutagenic. No preferential killing of the DNA repair-deficient strain.	--	Simmon 1978
Simazine	E. coli	Forward mutation to streptomycin resistance. Concentrations unspecified.	Nonmutagenic	--	Fahrig 1974
Simazine	E. coli	Forward mutation (Gal R^s). Concentration unspecified.	Nonmutagenic	--	Fahrig 1974
Simazine	Bacillus subtilis	Assay measures preferential killing of DNA repair-deficient strain (m45) over the repair proficient strain (H17). Cells treated with 1.0 mg per disc per plate.	Nonmutagenic. No preferential killing of the DNA repair-deficient strain.	--	Simmon 1978
Simazine	Saccharomyces cerevisiae	Assay measures mitotic recombination. Strain D3 treated with a 5% concentration.	Nonmutagenic. No increase in mitotic recombinants.	--	Simmon 1978

Appendix E (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Simazine	S. cerevisiae	Mitotic gene conversion. Concentrations unspecified.	Nonmutagenic	--	Fahrig 1974
Simazine	Drosophila melanogaster	Sex-linked recessive lethal test. Males were exposed by feeding to 3, 5, and 2,000 ppm.	Weakly mutagenic. Increase frequency of lethal mutations at 2,000 ppm.	--	Valencia 1981
Princep® 80W (80% Simazine)	D. melanogaster	Assays for dominant lethal mutations, sex-linked recessive lethal mutations, and chromosomal breakage, nondisjunction and loss were performed on males treated by injection or by larval feeding. In dominant lethal test, males were fed 0.40 or 0.60% or injected with 0.008%.	Significant increase in rate of apparent dominant lethals. Simazine elevated X-linked recessive lethals when injected but not when fed. No significant increase in partial loss of the Y chromosome or six chromosome nondisjunction was observed.	--	Murnik and Nash 1977
Simazine	Serratia marcescans	Back mutation. Concentrations unspecified.	Nonmutagenic	--	Fahrig 1974
Simazine	WI-38 cells	DNA repair synthesis assay. Simazine tested at concentrations of 10^{-7} to 10^{-3}M in absence of metabolic activation, and 10^{-5}M to 10^{-3}M in presence of metabolic activation provided by mouse liver homogenate.	No unscheduled DNA synthesis indicating no damage to DNA.	--	Simmon 1978

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Simazine

Appendix F

Summary of Metabolism Studies with Simazine

Formulation	Organism	Dose	Comments	References
Simazine (Technical)	Rainbow trout	1, 4, and 12.5 ppm (a.i.) in water, 24-hour exposure	Simazine residues in fish were 0.15 (±0.03), 1.03 (±0.18), and 3.40 (±0.29) ppm respectively. (Values in parentheses are standard errors of means.)	Dodson and Mayfield 1979
Princep® 80W	Rainbow trout	1, 4, and 12.5 ppm (a.i.) in water, 24-hour exposure	Simazine residues in fish were 0.08 (±0.01) 1.18 (±0.30), and 2.23 (±0.29) ppm respectively. (Values in in parentheses are standard errors of means.)	Dodson and Mayfield 1979
Simazine	Green sunfish	1.0 and 3.0 ppm, 3-week exposure	Simazine residues in fish were 0.95 and 2.29 ppm, respectively. No residues were found in fish after 7 days in fresh water. Most residues found in viscera.	Rogers 1970 in Dodson and Mayfield 1979 and in Mauck 1974
Simazine 80W	Fish (Bullhead, Bluegill, Green sunfish, Pumpkinseed sunfish and Goldfish)	16 acre pond treated with 1.7 ppm (a.i.) and fish sampled for 30 days.	Residues in fish declined during study period-residues in viscera from high of 1.1 ppm one day after application to 0.47 ppm 30 days after, similar pattern with fillets. Skin residues reached a high of 0.95 ppm on day 8 and a low of 0.56 ppm at 30 days.	Thomas 1967 in Mauck 1974
Simazine	"Fish"	Dose not given, fish sampled for 27 days.	Simazine residues in fish were generally lower than residues in the water from which the fish were taken.	Data from Fish Pesticide Research Laboratory (U.S. Department of the Interior), Columbia, Missouri cited in Mauck 1974

Appendix F (Concluded)

Formulation	Organism	Dose	Comments	References
Simazine 80W	Bluegill and Goldfish	5.0 ppm (a.i.) in concrete pools	Residues in fish below detectable limits, due either to lack of absorption or to rapid metabolism.	Unpublished study by P.A. Gilderhaus (U.S. Department of the Interior 1969) cited in Mauck 1974
Simazine	Bluegill and Catfish	Not given	Soluble residues in muscle of treated fish consisted of simazine and de-alkylated simazine metabolites.	Ciba-Geigy Chemical Corporation studies cited in Mauck 1974
Simazine	Rainbow trout	Fish placed in water with 2.5 ppm simazine, test medium exchanged on day 14, and replaced with fresh water on day 28.	Simazine residues maintained a constant level slightly above water concentration during first 28 days. In freshwater, tissue levels rapidly decreased to below detectable amounts. No adverse effects were noted.	Bathe et al. 1975
Simazine	Holstein dairy cows	Fed 5 ppm simazine (dissolved in acetone) based on 50 lb food daily, for 3 days.	No residues were found in milk. About 1% of intact simazine was collected in urine.	St. John et al. 1965
Simazine	Cows	80 ppm in total diet	0.01 to 0.02 ppm in milk.	U.S. Environmental Protection Agency registration data cited in Ghassemi et al. 1981
Simazine (^{14}C-labelled)	Rats	Not given	Small amounts ("few percent") recovered in feces and urine.	Unpublished Ciba-Geigy Chemical Corporation studies cited in Esser et al. 1975
Simazine (^{14}C-labelled)	Daphnids and Algae	0.1 to 10.0 ppm for up to 42 days	Organisms in static tests reached bioaccumulation equilibrium within one day. In flow-through systems, they continued to accumulate simazine gradually. Overall bioaccumulation levels were low.	Isensee and Yochim 1980

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Herbicide Background Statement: Triclopyr

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Triclopyr Summary

Major Trade Name: Garlon®

Chemical Name: [(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid.

Major Forestry Applications: Applied as a broad spectrum herbicide for timber management and engineering.

Detailed information on toxicology, environmental fate, and hazard assessment can be found in the body of the Herbicide Background Statement.

Toxicology

Triclopyr, as the triethylamino salt, is slightly toxic to practically nontoxic to invertebrates and microorganisms. In those fish tested, the butoxyethyl ester of triclopyr is highly toxic and the triethylamine salt and unformulated triclopyr are slightly toxic. Triclopyr and its formulations have low acute oral toxicities to birds with LD_{50}'s > 1,698 mg/kg.

In experimental studies with mammals, triclopyr causes slight to severe eye irritation, slight to moderate irritation, and nasal irritation. Tryclopyr is moderately toxic in acute dietary feeding studies at high doses, but causes no toxic effects in chronic dietary feeding at low doses. It causes no reproductive and teratogenic effects, but is mildly fetotoxic. There is no evidence for carcinogencity. Triclopyr is not mutagenic in bacterial systems, but is weakly positive in a dominant lethal assay in rats.

Environmental Fate

Triclopyr is absorbed by both plant leaves and roots and is readily translocated. It appears to interfere with normal plant growth processes. It is rapidly absorbed by animals and subsequently excreted by the kidney, mostly in unmetabolized form. Triclopyr does not bioaccumulate in fish.

In soil, triclopyr does not strongly adsorb and is potentially mobile. It is rapidly degraded by microorganisms and has a short persistence in soil environments. Rapid photodegradation is the major means by which triclopyr is degraded in aquatic environments.

Exposure and Hazard

The estimated occupational and environmental exposures in humans by dermal, oral, and inhalation routes should not result in adverse

toxic effects if proper care is taken during application of triclopyr. Triclopyr has not been shown to be toxic or to have reproductive, teratogenic, or mutagenic effects at exposures well in excess of those to which forest workers, visitors, and animal residents would be exposed.

1.0 General Information

Triclopyr, also known by the trade name* Garlon®, is a selective herbicide used for control of a variety of woody plants and broadleaf weeds. Triclopyr interfers with the normal growth response in plants. The precise physiology of the active ingredient is not known; however, the compound is absorbed readily through both roots and leaves and translocates both up and down the plant stem, accumulating in meristematic tissue. It is particularly effective in control of root-sprouting plant species (Weed Science Society of America 1983).

1.1 Normal Use Patterns

Triclopyr is a broad-spectrum herbicide originally developed for control of vegetation along utility rights-of-way and on industrial sites (McKellar et al. 1982). Triclopyr provides good control of most woody plants found in forest-site preparation areas. When applied as a foliar spray, it provides good control in noncrop areas. Noncrop applications include industrial, manufacturing, and storage sites, and pipeline, railroad, highway and electric transmission rights-of-way. It is effective in controlling species resistant to 2,4,5-T such as sassafras, black locust, ash, maple, blackberry and conifers. It also provides good control of poplar, aspen, birch, red and white oak, sumac, blackgum, sweetgum, hickory and willow. The triethylamine salt formulation (Garlon® 3A) is used to control woody plants when applied to cut surfaces by means of tree injection, or by girdle, frill, and stump sprays. The butoxyethyl ester formulation (Garlon®4) is used as a basal spray, either in an oil or oil-water spray solution (The Dow Chemical Company 1983a). It is also used in aerial applications.

Usual rates of application for triclopyr range from 0.25 to 9 lb/acre (0.28 to 10 kg/ha). Statistics on the use of triclopyr formulations by the USDA Forest Service are presented in Table 1-1. Application rates in 1982 by the USDA Forest Service ranged from 1.5 to 3 lb/acre for aerially applied Garlon®4 with 13 projects; from 0.5 to 5.2 lb/acre for 9 projects with ground-applied Garlon®4; and from 1.3 to 7.4 lb/acre for 6 projects with ground applied Garlon®3A (Gross 1983).

Triclopyr is manufactured, formulated, and marketed by The Dow Chemical Company. Triclopyr formulations are listed and described in Table 1-2. In addition to these formulations in which the only active ingredient is triclopyr, a formulation containing picloram and triclopyr is also available. This product, called M-4450, contains

*Trade names are used only to provide information and do not imply product endorsement.

Table 1-1

USDA Forest Service Use of Triclopyr

Purpose	Acid Equivalent/Per Acre (lbs) Range	Acid Equivalent/Per Acre (lbs) Average	Acres Treated	Formulation	Application Method
		1981			
Aerial					
Right-of-way	0.3-3.0	1.1	1,135	Liquid	Helicopter
Ground					
Conifer release	2.2	2.2	9	Liquid	Broadcast
Right-of-way	1.7	1.7	135	Liquid	Broadcast
Site preparation	2.9	2.9	30	Liquid	Broadcast
		1982			
Aerial					
Timber Management (13 projects)	1.5-3.0	2.2	2445	Garlon® 4	Helicopter
Ground					
Timber Management (9 projects)	0.5-5.2	1.9	567[a]	Garlon® 4	Ground Foliar and Basal Spray
Timber Management and Engineering (6 projects)	1.3-7.4	4.8	--	Garlon® 3A	Ground Foliar, Injection, and Cut Surface

[a]Includes both Garlon® 4 and Garlon® 3A combined.

Source: Gross 1983

Table 1-2

Triclopyr Formulations

Formulation*	Active Ingredient
Garlon® 3A	3 lb/gallon (0.36 kg/ℓ) acid equivalent as the triethylamine salt
Garlon® 4 (0.1 soluble, water emulsifiable)	4 lb/gal (0.48 kg/ℓ) acid equivalent as the butoxyethyl ester

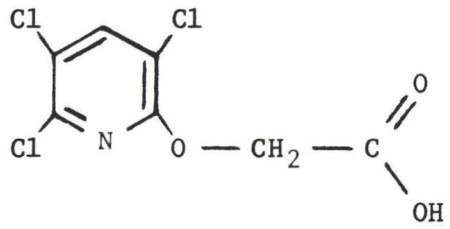
*Formulations including other active ingredients are omitted.

Source: The Dow Chemical Company 1983b and Weed Science Society of America 1983.

2 lb (0.24 kg) butoxyethyl ester of triclopyr and 1 lb (0.12 kg) isooctyl ester of picloram per gallon (per ℓ) of formulation (Weed Science Society of America 1983).

1.2 Physical and Chemical Properties

The active ingredient of triclopyr is [(3,5,6-trichloro-2-pyridinyl) oxy]acetic acid with the following structural formula (Weed Science Society of America 1983):



Triclopyr has a molecular formula of $C_7H_4Cl_3NO_3$ and a molecular weight of 256.48. In its pure form, triclopyr is an odorless white solid with a melting point of 298.4 to 302.0°F (148 to 150°C). Triclopyr is only slightly soluble in water, having a solubility of 430 ppm by weight (0.043%) at 77°F (25°C). With the exception of n-hexane (solubility of 410 ppm [0.041%] at 77°F [25°C]), triclopyr is more soluble in organic solvents than it is in water, having solubilities of 989,000 ppm (98.9%) in acetone, 126,000 ppm (12.6%) in acetonitrile, 2,730 ppm (2.73%) in both benzene and chloroform, 30,700 ppm (30.7%) in n-octanol, and 2,790 ppm (2.79%) in xylene at 77°F (25°C) (Weed Science Society of America 1983).

Triclopyr is readily degraded by both chemical and biological mechanisms, primarily photodegradation and microbial decomposition, respectively. In water and soil, the acid form of triclopyr is stable to hydrolysis, but the ester rapidly undergoes hydrolysis to the acid form. Amine formulations also convert rapidly to the acid form in both water and soil. The triclopyr acid that is formed in both soil and water is neutralized to a salt under normal environmental pH's (The Dow Chemical Company 1983b).

Formulations of triclopyr are combustible. The flash point for Garlon®3A is 110°F (43°C) and for Garlon®4 is 147°F (64°C) (The Dow Chemical Company, 1980, 1981). On extended exposure, there is some corrosion to aluminum by triclopyr formulations (Weed Science Society of America 1983).

2.0 Toxicity of Triclopyr

Triclopyr is a relatively new herbicide for which very limited toxicity data have been reported. The majority of the reported data are from the manufacturer, The Dow Chemical Company. The toxicological properties of triclopyr, summarized in Table 2-1, indicate that this herbicide and its formulations have a wide range of toxicity to those organisms tested. Studies with mammals indicate that triclopyr and its formulations induce low to moderate acute toxicity, mild subchronic toxicity, and no chronic toxicity. In limited studies in rodents, no carcinogenic activity was observed. Triclopyr is mildly fetotoxic but is not teratogenic. Although nonmutagenic in bacterial and cytogenetic assays, triclopyr is weakly positive in rat, but not mouse, dominant lethal assays. Skin and eye irritation appear to be the human health hazards of concern with Garlon® 3A, but Garlon® 4 is nonirritating (Weed Science Society of America 1983).

2.1 Invertebrates and Microorganisms

The few data available on invertebrate and microorganism toxicity, primarily for the triethylamine salt (Garlon®3A) and generated by The Dow Chemical Company, are presented in Appendix A. These data indicate that the triethylamine salt of triclopyr is only slightly toxic to practically nontoxic to organisms tested, with a 96-hour LC_{50} of 895 ppm in shrimp, of >1,000 ppm in crabs, and a 48-hour LC_{50} between 56 and 87 ppm in oysters (Dow Chemical Company data reported in Ghassemi et al. 1981 and in Weed Science Society of America 1983). In Daphnia magna, the 48-hour acute LC_{50} was 1,170 ppm, and the 21-day chronic LC_{50} was 1,140 ppm for the triethylamine salt of triclopyr (Gersich, et al. in press). Hallborn and Bergman (1979 in Ghassemi et al. 1981) report no significant effects of Garlon®3A on lichen nitrogen fixation. Triclopyr® at 500 ppm had no apparent effect on the growth of six species of soil microorganisms after 72-hours incubation (U.S. Environmental Protection Agency registration data reported in Ghassemi et al. 1981). In aquatic organisms, LC_{50}'s of greater than 10 ppm are considered indicative of only slight toxicity (Clarke et al. 1970).

Toxicity studies with invertebrates and microorganisms have been conducted in controlled laboratory environments. As a consequence of the laboratory environment and the experimental designs employed, the results do not necessarily present a true reflection of the potential effects of triclopyr in a natural environment. For example, in the laboratory, aquatic organisms were kept for as long as 4 days in static conditions with continuous exposure to triclopyr. Microorganisms were grown in culture media in the laboratory with triclopyr

Table 2-1

Summary of Toxicological Properties of Triclopyr

1. Acute Oral Toxicity

Organism (Formulation)	Adverse Effects
Rat (Triclopyr)	LD_{50} = 630 to 729 mg/kg
Rat (Garlon® 4)	LD_{50} = 2,000 to 2,500 mg/kg
Rat (Garlon® 3A)	LD_{50} = 2,000 to 3,000 mg/kg
Rabbit (Triclopyr)	LD_{50} = 550 mg/kg
Guinea Pig (Triclopyr)	LD_{50} = 310 mg/kg
Mallark Duck (Triclopyr)	LD_{50} = 1,698 mg/kg
Mallard Duck (Garlon® 4)	LD_{50} = 4,640 mg/kg
Mallard Duck (Garlon® 3A)	LD_{50} = 3,176 mg/kg
Fish (Garlon® 4)	LC_{50} = 0.74 to 0.87 ppm
Fish (Triethylamine Salt and Garlon® 3A)	LC_{50} > 100 ppm

2. Acute Dermal Toxicity

Organism (Formulation)	Adverse Effects
Rabbit (Triclopyr)	LD_{50} >2,000 mg/kg
Rabbit (Garlon® 4)	LD_{50} >4,000 mg/kg
Rabbit (Garlon® 3A)	LD_{50} >4,000 mg/kg

3. Inhalation Toxicity

Organism (Formulation)	Adverse Effects
Rat (Triclopyr)	No adverse effects.
Rat (Garlon® 4)	Caused nasal irritation only.

Table 2-1 (Continued)

4. Eye Irritation

Organism (Formulation)	Adverse Effects
Rabbit (Triclopyr)	Slightly irritating.
Rabbit (Garlon® 4)	Not an eye irritant.
Rabbit (Garlon® 3A)	Severely injurious.

5. Subchronic Oral Toxicity

Organism (Formulation)	Length of Treatment	Dose Tested	Adverse Effects
Rat (Triclopyr)	90 days	3, 10, 30 and 100 mg/kg/day in diet	Decreased body weight, decreased liver weight and increased kidney weight in males at 100 mg/kg/day.
Mouse (Triclopyr)	90 days	6, 20 and 60 mg/kg/day in diet	Reduced liver weight in males at 60 mg/kg/day. mg/kg/day.
Dog (Triclopyr)	183 days	2.5 mg/kg/day in diet	Slightly reduced kidney excretion.
Dog (Triclopyr)	228 days	5, 10 and 20 mg/kg/day in diet	Decreased weight gain and food consumption. Liver and kidney effects developed due to urinary retention of Triclopyr.
Monkey (Triclopyr)	Unspecified	20 and 30 mg/kg/day in diet	No adverse effects.

Table 2-1 (Concluded)

6. Reproduction, Teratology and Fertility

Organism (Formulation)	Dose and Length of Treatment	Adverse Effects
Rat (Triclopyr)	Three generation feeding study at doses of 3, 10 and 30 mg/kg/day	No reproduction or teratogenic effects; no adverse effects on growth or maturation.
Rat (Triclopyr)	Gavaged pregnant rats with 50 to 200 mg/kg/day on days 6-18 of gestation.	Mild fetotoxicity-delayed ossification of skull in offspring of pregnant rats receiving 200 mg/kg/day
Rabbit (Triclopyr)	Gavaged pregnant rabbits with 10 or 20 mg/kg/day on days 6-18 of gestation.	Not teratogenic at 100 mg/kg/day
Rabbit (Triclopyr)	Gavaged pregnant rabbits with 25, 50 or 100 mg/kg/day on days 6-18 of gestation.	Increased mortality at all doses.

7. Chronic Toxicity

Organism (Formulation)	Dose of Length of Treatment	Adverse Effects
Rat (Triclopyr)	Fed 3, 10 or 30 mg/kg/day for 2 years	No toxic effects. Not carcinogenic.
Mouse (Triclopyr)	Fed 3, 10 or 30 mg/kg/day for 2 years	No toxic effects. Not carcinogenic.

8. Mutagenicity

Organism (Formulation)	Adverse Effects
Bacterial assay systems (unspecified)	Nonmutagenic
Cytogenetic assays (unspecified)	Nonmutagenic
Dominant lethal studies in rats and (unspecified)	Weakly positive in rats; negative in mice.

[a] Toxicology data are extracted from Appendices A through E. Source references can be found in the appropriate Appendix.

at concentrations greater than those normally found in the environment following application. In contrast, in natural aquatic and nonsoil terrestrial environments, exposure to triclopyr or other man-made chemicals is usually a transient phenomenon whose duration depends on the rate of streamflow or on the rate of decomposition of the chemical. Triclopyr is readily degraded by light and by microbial action. Additionally, in natural systems, interaction with other man-made and naturally occurring chemicals, and changes in environmental factors, may affect herbicide assimilation and metabolism by invertebrates and microorganisms. These interactions, in turn, potentially alter the tolerance levels of organisms to the herbicides.

2.2 Fish

The few available studies of the toxicity of triclopyr and its formulations to fish, summarized in Appendix B, indicate a wide response of fish to the two triclopyr formulations and to unformulated triclopyr. Clarke et al. (1970) considers chemicals with LC_{50}'s less than 1 ppm to be highly toxic and those with LC_{50}'s greater than 10 ppm to be slightly toxic to fish. Based on these criteria, Garlon®4, the butoxyethyl ester of triclopyr, is highly toxic to both rainbow trout and bluegill with 96-hour LC_{50}'s of 0.74 and 0.87 ppm, respectively (The Dow Chemical Company 1983a). The triethylamine salt, formulated as Garlon®3A, is only slightly toxic to rainbow trout and to bluegill with 96-hour LC_{50}'s of 552 and 891 ppm, respectively (The Dow Chemical Company 1983a). Similarly, unformulated triclopyr, with reported 96-hour LC_{50}'s of 117 and 148 ppm in rainbow trout and bluegill, respectively, is only slightly toxic to these species (The Dow Chemical Company 1983a). In a study with fathead minnows, Mayes et al. (in press) reported a 96-hour static LC_{50} of 245 ppm and for flow-through tests, 96-hour and 192-hour LC_{50}s of 120 and 101 ppm, respectively. These data are substantiated for Garlon®3A in a report by Johnson and Finley (1980) in which rainbow trout and bluegill both were found to have 96-hour LC_{50}'s greater than 100 ppm.

It should be noted that in natural soil and aquatic environments, both amine and ester formulations rapidly convert to triclopyr acid, which in turn is neutralized to a salt at normal environmental pH. Thus, for both Garlon®3A and Garlon®4, there is rapid conversion to the same salt (The Dow Chemical Company 1983b). No information is provided with any of the fish toxicity data on the actual form of triclopyr present in the test water or on decomposition or other reactions that may have occurred to affect the parent formulation used in the study. Triclopyr formulations are rapidly photodegraded in water, however.

2.3 Birds

The toxic effects of triclopyr have been investigated in birds in a small number of studies conducted by The Dow Chemical Company. These studies are summarized in Appendix C. Triclopyr and its formulations have a low acute oral toxicity to mallard ducks, with LD_{50}'s of 1,698, 3,176, and 4,640 mg/kg body weight for triclopyr, Garlon® 3A, and Garlon® 4, respectively (Dow Chemical Company data reported in Weed Science Society of America 1983). Eight-day subchronic feeding studies have been conducted for mallard ducks and both bobwhite and Japanese quail with triclopyr, and for mallard ducks and bobwhite quail with Garlon® 3A and Garlon® 4 (The Dow Chemical Company 1983a). Subchronic oral toxicity of triclopyr and its formulations is low in these species, with LC_{50}'s from 2,935 to greater than 5,000 ppm for unformulated triclopyr, LC_{50}'s greater than 10,000 ppm for Garlon® 3A, and LC_{50}'s greater than 9,000 ppm for Garlon® 4. No field studies on the toxic effects of triclopyr or its formulations in birds have been reported.

2.4 Mammals

The potential health hazards from exposure to triclopyr have been evaluated in laboratory animals including rats, mice, rabbits, dogs, guinea pigs, and monkeys. The limited published toxicity data available from these studies are described below and summarized in Table 2-1, Appendix D, and Appendix E.

2.4.1 Acute Toxicity

Acute toxicity data have been reported from oral, dermal, and inhalation studies. Brief descriptions with no data have been reported from eye irritation tests.

2.4.1.1 Oral. The acute oral toxicity of triclopyr is moderate, as seen in Table 2-1. In male rats, the LD_{50} was 729 mg/kg, and in female rats the LD_{50} was 630 mg/kg (The Dow Chemical Company 1983b). Formulated Garlon® 4 and Garlon® 3A have even lower acute toxicities than triclopyr, with LD_{50}'s in the range of 2,000 to 2,500 mg/kg and 2,000 to 3,000 mg/kg, respectively. All of the LD_{50}'s are in toxicity category III as classified by the U.S. Environmental Protection Agency, warranting handling of triclopyr and its formulations with caution. The acute oral toxicity of triclopyr is also low in species other than rats. In rabbits, the LD_{50} was 500 mg/kg, and in guinea pigs the LD_{50} was 310 mg/kg (Weed Science Society of America 1983). No experimental details elucidating doses, route of administration, length of treatment, or adverse effects are available.

2.4.1.2 Dermal. Triclopyr and its formulations induce slight to moderate degrees of skin irritation. Studies with triclopyr, Garlon®3A, and Garlon®4 have not resulted in the determination of specific dermal LD_{50}'s. It has been reported that in rabbits the LD_{50} is greater than 2,000 mg/kg for triclopyr and greater than 4,000 mg/kg for both Garlon®4 and Garlon®3A (The Dow Chemical Company 1983b). However, no details have been reported for these dermal studies. Garlon®4 is slightly irritating to the skin after repeated and prolonged skin contact in rabbits. The undiluted form of Garlon®3A is slightly to moderately irritating to the skin (The Dow Chemical Company 1983b). Dilution of Garlon®3A with water to a 1:7 ratio is essentially nonirritating. This is the highest concentration labeled for ground application. A derivative of triclopyr, 2-butoxyethyl-((3,5,6-trichloro-2-pyridinyl)oxy) acetate, caused skin sensitization in 1 of 9 guinea pigs tested (Rao et al. 1981). No data are available on triclopyr skin sensitization potential.

2.4.1.3 Inhalation Toxicity. Table 2-1 summarizes two inhalation toxicity studies in rats. In one study, rats exposed for one hour to 5.34 ppm triclopyr developed no adverse effects (Weed Science Society of America 1983). In the second study, rats were exposed for four hours to an aerosol of Garlon®4 at a concentration of 0.82 ppm and particle size of ten microns or less. This caused no deaths. Nasal irritation was the only adverse effect reported (The Dow Chemical Company 1983b). No further data were available regarding these studies.

2.4.1.4 Eye Irritation. Although no data are available to report in Table 2-1 or Appendix D, a few summary statements concerning eye irritation have been made (The Dow Chemical Company 1983b). Triclopyr is slightly irritating to the eyes. Garlon®4 is not an eye irritant in rabbit tests. In contrast, Garlon®3A is severely injurious to the eyes. The Dow Chemical Company (1983b) states that "goggles are recommended during handling or use of Garlon®3A before dilution".

2.4.2 Subchronic Toxicity.

2.4.2.1 Oral. Triclopyr, provided in the diets of rats, mice, dogs, and monkeys, caused mild toxic effects at high-doses and no adverse effects at lower doses (The Dow Chemical Company 1983b and Weed Science Society of America 1983). Four studies have been partially described in the literature and are summarized in Table 2-1. In the first, male and female rats were fed triclopyr in doses including 3, 10, 30 and 100 mg/kg/day for 90 days. Decreased body weight, decreased liver weight, and increased relative kidney weights were observed in males at 100 mg/kg/day. No adverse effects

developed in males with doses equal to or less than 30 mg/kg/day or in females with doses equal to or less than 100 mg/kg/day.

Similarly, in the second study, mice of both sexes were fed diets with triclopyr in doses including 6, 20 and 60 mg/kg/day for 90 days. A slight reduction in relative liver weight at 60 mg/kg/day, and no adverse effects at lower doses, were observed in males. No toxic effects were observed in females at the three doses.

In a third study, beagle dogs were provided dietary triclopyr at doses of 5, 10 and 20 mg/kg/day for 228 days. Decreased body weight gain and food consumption were observed. Liver and kidney effects developed that were not described but were attributed to increased urinary retention of triclopyr. Beagle dogs were also fed 2.5 mg triclopyr/kg/day for 183 days. With this lower total dose, the only adverse effect reported was a slight reduction in kidney excretion determined by means of PSP dye excretion tests (The Dow Chemical Company 1983b).

The last study reported in Table 2-1 shows no evidence for sub-chronic toxicity, including no effect on renal excretion by dietary triclopyr in monkeys at doses of 20 or 30 mg/kg/day.

2.4.3 Chronic Toxicity.

Two separate feeding studies with rats and mice showed no chronic toxicity of triclopyr, as shown in Table 2-1. The animals were fed daily doses of 3, 10 and 30 mg/kg/day in the diet for approximately two years. Triclopyr induced no toxic effects in either rats or mice (The Dow Chemical Company 1983b).

2.4.4 Reproductive and Teratogenic Effects.

On the basis of three studies, summarized in Table 2-1, triclopyr has no adverse effect on reproduction, is not teratogenic in rats or rabbits, but is mildly fetotoxic. A three-generation feeding study at doses of 3, 10 and 30 mg/kg/day was conducted in rats. No teratogenic effects or adverse effects on reproduction, growth, or maturation were observed. Two studies in rabbits confirmed the non-teratogenicity of triclopyr at 25, 50 and 100 mg/kg/day. Mild fetotoxicity was observed in another study, in which pregnant rats were administered 25 to 200 mg/kg/day during organogenesis. The period of administration and route were not specified. Delayed ossification of the skull occurred in the offspring of pregnant rats treated with 200 mg/kg/day (The Dow Chemical Company 1983b and Hanley, et. al. in press).

2.4.5 Carcinogenicity

The two-year chronic studies described in Table 2-1 indicate no chronic toxic effects or carcinogenicity of triclopyr (The Dow Chemical Company 1983b). Two years is usually a sufficiently long treatment period in rats and mice for tumor development to occur if an appropriately high dose is tested. The highest dose tested in these studies was 30 mg/kg/day.

2.5 Mutagenicity

Triclopyr has been tested for mutagenicity in a variety of test systems and found to be weakly positive only in one, the dominant lethal study in rats. Triclopyr was nonmutagenic in bacterial assay systems, cytogenetic assays, and mouse dominant lethal studies (The Dow Chemical Company 1983b).

3.0 Environmental Fate

Triclopyr is effective against a wide variety of woody plants as a foliar spray, as a basal spray, and when applied to cut surfaces. It degrades rapidly in soils under environmental conditions that favor microbial growth (reported half-lives up to about 46 days); is rapidly degraded, primarily by photolysis, in water; and is readily eliminated by animals when ingested. Triclopyr is absorbed by both plant leaves and roots and is readily translocated throughout the plant. It induces an auxin-type response in growing plants in that it appears to interfere with the normal growth process. Thus, maximal plant response, particularly to foliar application, occurs when application occurs soon after full leaf development and when there is sufficient soil moisture for plant growth (The Dow Chemical Company 1983a and 1983b).

3.1 Bioaccumulation and Metabolism

No data are currently available on metabolism of triclopyr in plants. However, measurements of triclopyr residues in plants following treatment have been reported from Finland for triclopyr applied to fields (Siltanen et al. 1981) and for greenhouse-grown plants in Texas (Bovey et al. 1979 and Bovey and Mayeux 1980). Information on the metabolism and bioaccumulation of triclopyr in animals is derived from published product literature and U.S. Environmental Protection Agency registration data (reported in Ghassemi et al. 1981) generated by The Dow Chemical Company.

3.1.1 Plants

Triclopyr is taken up by plants through both roots and foliage and is then readily translocated throughout the plant. Translocation rates are affected by photoperiod and temperature. Radosevich and Bayer (1979 in Ghassemi et al. 1981) reported a maximum rate of translocation in a variety of plants under conditions of warm temperatures and long photoperiod days. Translocation was greatly reduced during short photoperiod days and colder temperatures. King and Radosevich (1979 in Ghassemi et al. 1981) found that greater amounts of triclopyr were absorbed by immature leaves of tanoak (*Lithocarpus densiflorus*) than by mature leaves. They also reported that surfaces containing large densities of stomata absorbed more triclopyr than surfaces of leaves containing lower stomate densities. These data indicate a possible relationship between rate of plant metabolic activity (increased photosynthesis with higher temperatures, longer photoperiod, and higher rates of gas exchange associated with greater stomate density) and absorption of triclopyr, although no direct correlations have been drawn.

No information on actual metabolism of triclopyr in plants is currently available. Residue levels have been measured following application of triclopyr in field and greenhouse studies, however.

Siltanen et al. (1981) reported on residue levels of triclopyr in cowberries, bilberries, and lichen following application of Garlon® (specific formulation not given) on small experimental field plots in Finland. For application rates of 0.22 to 2.01 lbs active ingredient per acre (0.25 to 2.25 kg/ha), residues were 0.3 to 1.9 ppm (by weight) in cowberries at 30 days post-treatment, 0.2 to 4.0 ppm in bilberries at 8 days post-treatment, and 0.15 to 1.7 ppm in lichens at 13 months post-treatment. In all cases, increasing application rates resulted in increased levels of residue.

Triclopyr was applied once to cowberries at a rate of 0.67 lb active ingredient per acre (0.75 kg/ha) in mid-June, July, August or September, and the berries were harvested in late September, such that intervals of 6 to 98 days existed between treatment and harvesting. Residues in the cowberries declined with residence time. These residues were 2.4 ppm 6-days post-treatment (September treatment), 0.7 to 1.1 ppm 30 to 36 days post-treatment (August treatment), 0.2 ppm 64 to 70 days post-treatment (July treatment), and 0.2 to 0.3 ppm 92 to 98 days post-treatment (June treatment). In bilberries similarly treated with 0.67 lb/acre (0.75 kg/ha) in mid-June, July, or August and harvested in late August, residues were 0.9 ppm (7 days post-treatment) to 0.7 ppm (69 days post-treatment) with no relationship between residence time and residue levels. It is unclear whether the differences in residue levels in cowberries is due to the time interval between treatment and harvest, or due to the environmental conditions, plant metabolism, and growth stage at the time of treatment with triclopyr (Siltanen et al. 1981).

Residues of triclopyr in greenhouse-grown honey mesquite were measured 3, 10, and 30 days following application of the ethylene glycol butyl ether ester or triethylamine salt of triclopyr to foliage, to soil, or to both foliage and soil (Bovey and Mayeux 1979). The triclopyr was applied at a rate equivalent to 1 lb/acre (1.1 kg/ha). Maximum residues of 28 ppm were found in leaves 10 days after foliar application of the amine salt. Maximum stem residues of 3.6 ppm triclopyr were found 10 days after foliar application of both the ester and the amine salt. In general, foliar applications of triclopyr resulted in higher leaf and stem residues, while soil applications resulted in higher root residues.

Bovy et al. (1979) measured triclopyr residues in greenhouse-grown huisache (Acacia farnesiana) following application of the triethylamine salt at rates equivalent to 1 and 2 lb/acre (1.12 and 2.24 kg/ha) as soil, foliar, and soil-plus-foliar

treatments. Maximum residues of 37 ppm were found in the stems immediately after foliar application of 1 lb/acre and 3 days after 2 lb/acre application. Maximum residues of 37 ppm were also found in stems immediately after soil-plus-foliar application at 1 lb/acre. In general, highest residue levels were in the stem and intermediate levels were in foliage for both foliar and soil-plus-foliar applications. Highest levels occurred in roots for soil applications.

3.1.2 Animals

The available information on the metabolism and bioaccumulation of triclopyr in animals is derived from data originated by The Dow Chemical Company. Triclopyr is rapidly absorbed by animals and is then rapidly excreted via the kidney, mostly as the parent compound. Small quantities of two other compounds, the metabolite trichloropyridinol or a conjugated form of the parent, triclopyr acid, are also excreted. At high doses, an increasing amount of excretion of triclopyr occurs through the feces (The Dow Chemical Company 1983b). Animal metabolism studies with triclopyr are summarized in Appendix F.

Rats dosed intravenously with 5 and 100 mg triclopyr per kg body weight excreted 91 and 83 percent, respectively, of the total dose via the kidneys. Dogs dosed with 0.5 and 20 mg radiolabeled triclopyr (route of administration not given) had half-lives for blood plasma clearance of 14 and 96 hours, respectively. These results indicate that triclopyr is rapidly cleared from animal systems, primarily via the kidneys. Residue studies with radiolabeled triclopyr administered to rats, dogs, and monkeys indicated higher concentrations of residues in the kidney than in blood plasma in dogs, in the liver and in fatty tissues compared to blood plasma in rats, and not in any of the tissues from the monkey. Bioaccumulation of triclopyr or of its metabolites was not observed in catfish or in mosquito fish.

3.2 Soil

Limited data are available on the behavior of triclopyr in soil. Much of the available information has been summarized by Ghassemi et al. (1981). In addition, recent studies have been conducted with forest soils in Sweden (Torstensson and Stark 1982) and in soils in West Virginia (McKellar et al. 1982). Triclopyr has a short persistence in soil. It is not strongly adsorbed to soil particles and therefore is potentially mobile in soil. The lack of persistence of triclopyr in soil is due to rapid degradation of the compound by soil microorganisms (The Dow Chemical Company 1983b). Although there is no direct evidence of photodegradation of triclopyr in soils, its rapid photolysis in water indicates that, when on the

surface of soils, it is likely to photodegrade. Because of its low volatility, volatilization of triclopyr should not be a major means by which triclopyr is lost from the soil (Ghassemi et al. 1981).

Although triclopyr is generally considered to have a short persistence in soils, the actual half-life is strongly dependent on specific soil type and climatic conditions. McKellar et al. (1982) estimated the half-life of triclopyr residues in soil in a small watershed in West Virginia to be between 14 and 16 days. However, much of the aerially applied triclopyr (Garlon® 3A applied at a rate of 10 lb/acre [11.2 kg/ha]) was intercepted by foliage. Average triclopyr residues in soil from the treated area in this study, measured on the day of treatment, were nondetectable in densely wooded areas, 4.4 ppm in lightly wooded areas, and 18 ppm in open areas. Residues in soil from the latter two areas declined to <1 ppm within 4 weeks and were nondetectable at 28 weeks and at subsequent sampling periods. The Dow Chemical Company (1983a) reports a half-life of 10 days in a silty clay loam and 46 days in a loam. The loam used in this latter study was maintained in the laboratory at 95°F (35°C) with moisture at field capacity. Dow (The Dow Chemical Company (1983b) reports the average half-life of triclopyr in soil to be 30 days. Laskowski et al. (1982) report a soil half-life of 40 days for triclopyr. An average half-life of 46 days is reported in Weed Science Society of America (1983) and in Ghassemi et al. (1981).

In a field study in Sweden, Torstensson and Stark (1982) applied 2.0 lb Garlon® 3A active ingredient/acre (2.2 kg/ha) and 1.7 lb Garlon® 4 active ingredient/acre (1.9 kg/ha) to 8 different forest soils. Residues of triclopyr persisted for 1 to 2 years, and in some cases in excess of 2 years, at levels approximately 10 percent or less of residues sampled immediately after application. It should be noted, however, that measured summer soil temperatures were usually only 55.4°F (13°C) or less and never more than 57.2°F (14°C). These temperatures are not particularly favorable to microbial degradation.

Ghassemi et al. (1981) reported Dow Chemical Company data on soil residues of triclopyr metabolites. The half-life of tricloropyridinol was 8 to 279 days in 15 soils. In 12 of these soils it was less than 90 days. Trichloromethoxy pyridine, which degraded to carbon dioxide, had a half-life of 50 days in two soils, and a half-life of greater than 300 days in another soil.

Triclopyr rapidly adsorbs onto soil particles. The degree of adsorption is dependent on soil type, with soil organic matter the primary parameter involved. Increased soil organic matter results in increased adsorption of triclopyr. A major degradation product of triclopyr, trichloropyridinol, is less mobile than the parent triclopyr (Dow Chemical Company data reported in Ghassemi et al. 1981).

The ability of triclopyr to be leached through any given soil is a function of the adsorption capacity of the soil. Limited data from the few studies conducted to date indicate that triclopyr can be leached from soil, but that the rate of leaching would be reduced in soils with high organic matter content compared to soils with a low organic matter content. In a soil composed of loam and sand with low amounts of organic matter (0.62% organic carbon), 75 to 80 percent of applied triclopyr leached through a 12-inch column between days 11 and 15 when 0.5 inch of water was applied daily. The trichloropyridinol metabolite required twice as much water to leach through the soil than did the parent triclopyr. Leaching of triclopyr and its two metabolites, trichloropyridinol and trichloromethoxy pyridine, was studied in six soils in various parts of the United States following field application of Garlon®3A at a rate of 3 gal./acre under conditions of normal rainfall. Small amounts of triclopyr and the metabolites were observed in the 6 to 12 inch and in the 12 to 18 inch soil layers 4 to 8 weeks after application. The trichloropyridinol degradate leached less than the parent triclopyr, with maximum residues in the 0 to 6 inch layer at 4 to 8 weeks. Residue levels for this compound subsequently declined. Trichloromethoxy pyridine maintained a concentration of 0.1 ppm or less in all soil layers sampled (Dow Chemical Company data reported in Ghassemi et al. 1981).

Rainfall relatively soon after application of triclopyr may result in runoff loss into watersheds due to material being desorbed from soil particles and carried in solution, or carried adsorbed onto eroded sediment (Ghassemi et al. 1981). Monitoring studies of runoff in a small Oregon watershed found residues of 6 ppb in the runoff water 5 months after treatment with 3 lb/acre (3.36 kg/ha) of triethylamine salt of triclopyr and approximately 59 inches of natural rainfall (Norris et al. 1976 summarized in EPA registration data reported by Ghassemi et al. 1981).

Microbial degradation is the primary mechanism by which triclopyr is rapidly degraded in soils to two metabolites, 3,5,6-trichloro-2-pyridinol (referred to here as trichloropyridinol) and trichloro-methoxy pyridine (Dow Chemical Company reports cited in Ghassemi et al. 1981). Incubation studies with radiolabeled triclopyr under aerobic conditions indicated degradation to trichloropyridinol with a half-life of 79 to 156 days at 50°F (15°C) and less than 50 days at 77 to 95°F (25 to 35°C). Trichloropyridinol is then degraded to trichloromethoxypyridine and carbon dioxide. Trichloropyridinol is also a degration product of trichloromethoxy pyridine. Under anaerobic conditions, such as in waterlogged soil, microbial degradation of trichlopyr still continues but is slowed by as much as 5 to 8 times (Dow Chemical Company reports cited in Ghassemi et al. 1981).

Triclopyr in soil is not thought to be degraded to any great extent by chemical hydrolysis and, due to its low volatility, is not thought to volatilize from soil to any appreciable extent. Since it does photodegrade in water fairly readily, triclopyr on light-exposed leaf and soil surfaces may also photodegrade rapidly (Ghassemi et al. 1981).

3.3 Water

Laboratory studies indicate that photodegradation is the major means by which triclopyr is degraded in aquatic environments. Photodegradation in water is rapid, with a half-life as short as 10 hours at 77°F (25°C) (Weed Science Society of America 1983). The major degradation product of triclopyr is trichloropyridinol, which is then further degraded. Trichlopyr is stable to hydrolysis for up to 9 months at pH of 5, 7, and 8 in buffered solutions at temperatures from 59 to 95°F (15 to 35°C) (Dow Chemical Company report cited in Ghassemi et al. 1981). The ester form of triclopyr, however, is rapidly converted in water by hydrolysis to the acid form (The Dow Chemical Company 1983b).

McKellar et al. (1982) monitored residues of triclopyr and trichlorpyridinol in streamflows draining a small West Virginia watershed that had received application of 10 lb/acre (11.2 kg/ha) Garlon®3A by helicopter. Water samples were taken periodically from two streams running through the target area. Sample sites were 100 feet downslope from the treated area for each stream, with a third sample site at a weir where the two streams joined. Water samples were collected for up to 510 days after treatment. Residues in these samples ranged from nondetectable to 0.02 ppm at the weir (about 200 feet from the treatment area) and in one stream, and from nondetectable to 0.08 ppm in the second stream. No residues of trichloropyridinol were detected in any water samples during the course of this study.

4.0 Exposure and Hazard Assessment

Forest workers, visitors, and resident or transient animals are all potentially exposed to triclopyr during forest spray operations. For humans and other terrestrial animals, exposures can be by dermal, inhalation, and oral (ingestion) routes. Aquatic animals are potentially exposed by immersion in waterbodies that may contain dissolved triclopyr. Calculations of exposure to triclopyr in this section follow the assumptions and methodology detailed in Section 4.0 of the General Introduction to the Herbicide Background Statements. In general, it should be noted that environmental exposures to triclopyr are brief, and that any area sprayed in a given year may well not be sprayed again for several years. Occupational exposures, at least for some individuals, may be of longer duration than environmental exposures. However, only a limited number of individuals are so exposed.

4.1 USDA Forest Service Triclopyr Application

Available application data for triclopyr used by the USDA Forest Service for 1982 are presented in Table 4-1. Actual exposure to triclopyr differs among Forest Service personnel involved in herbicide use, other Forest Service personnel, visitors to Forest Service lands that have been treated with herbicides, and wildlife species that are permanent or transient inhabitants of treated areas.

The data presented in Table 4-1 indicate the number of forest workers involved in application of triclopyr in 1982, as well as the duration of exposure on a daily and an annual basis. However, the actual exposure of these workers depends not only upon the specific formulation, method, and rate of application, but also on the protective measures employed, the operating condition of the equipment used, and accidental events which expose workers to the pesticide.

Triclopyr has a short persistence in soil, is not strongly adsorbed to soil particles and is rapidly degraded. In water, triclopyr is readily degraded by light and by microbial action. Consequently, the potential for incidental exposure of humans and wildlife to triclopyr is low, existing only for a very short period immediately after its application.

4.2 Exposure

Estimates of potential exposure to triclopyr are based on aerial as well as ground application. The maximum aerial application rate for triclopyr (see Table 1-1) is 3.0 lb a.i./acre, while the compound

Table 4-1

USDA Forest Service Application Data for Triclopyr (1982)

Formulation (Number of Projects)	Method of Application	Average Number People Exposed/Project	Total People Exposed[a]	Duration of Exposure				Type of Management (Projects)
				Average		Maximum		
				Hrs/Day	Days/Yr	Hrs/Day	Days/Yr	
Garlon® 3A (6)	Ground Foliar, Injection Cut Surface	4.7	28	4.8	10	5	9.8	Timber (4) Engineering (2)
Garlon® 4 (9)	Ground Foliar and Basal Spray	5	45	5.1	5.1	5.6	7.4	Timber (9)
Garlon® 4 (13)	Aerial	7.5	98	2.9	4.7	4.3	5.8	Timber (13)

[a]Grand Total = 171

Source: Gross 1983

is applied from the ground (ground foliar) at rates as high as 7.4 lb a.i./acre. In both aerial application and ground foliar application, triclopyr is applied as a liquid. Therefore, in this analysis, for the calculations in Table 4-2 that are based on aerial application (e.g., spray/observer dose, wildlife dermal exposures, and water concentrations), an application rate of 3.0 lb a.i./acre is assumed. Those calculations in Table 4-2 that are based on ground spraying (e.g., backpack sprayer dose and wildlife oral exposures) assume an application rate of 7.4 lb a.i./acre. Other specific assumptions, based upon the toxicological and chemical characteristics of triclopyr and on its behavior in the environment, are discussed in the following sections. Table 4-2 summarizes the estimates for occupational and environmental doses and exposures by means of immersion, dermal, inhalation, and oral routes.

4.2.1 Occupational Doses.

Data are not available on the daily occupational exposures or doses of triclopyr to forestry workers. However, estimates of occupational doses have been derived based on the urinary output of several categories of workers exposed to phenoxy herbicides (e.g., 2,4-D and 2,4,5-T). As indicated in the General Introduction to the Herbicide Background Statements, urinary output was used to calculate total doses (i.e., regardless of route) to workers exposed to phenoxy herbicides, and this quantity was then expressed on the basis of an application rate of 1 lb/acre. Daily occupational dose estimates for triclopyr are based on exposures on a per pound per acre application rate multiplied by 3.0 lb/acre for observers and 7.4 lb/acre for backpack sprayers. In the case of the aerial spray observer, dose estimates are based upon a maximum of one daily exposure to direct aerial spray with an unprotected skin surface area of 2 square feet (see the General Introduction to the Herbicide Background Statements). The maximal herbicide dermal absorption rate is assumed to be, in the absence of data to indicate otherwise, 10 percent. At 3.0 lb a.i./acre aerial application rate, the following estimate of daily dose is derived:

Observer with Direct Spray Deposition: up to 0.12 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

For ground applications at 7.4 lbs/acre application rates, the following estimate of daily dose is derived:

Backpack Sprayer: 0.296 mg/kg (0.04 mg/kg at 1 lb/acre application rate).

Occupational doses for the mixer/loader are assumed to be independent of application rate and have been estimated to be 0.1 mg/kg.

Table 4-2

Summary of Occupational Dose, Environmental Exposure, and Toxicity for Triclopyr

Occupational Dose	Exposure Route[a]		
	Dermal	Inhalation	Oral
Mixer/Loader	0.1 mg/kg		[b]
Observer	up to 0.12 mg/kg		
Backpack Sprayer	0.296 mg/kg		
Environmental Exposure			
Fish	0.03 ppm (Immersion)		[c]
Rabbits	1.209 mg/kg	Negligible	7.77 mg/kg
Deer	0.303 mg/kg	Negligible	10.66 mg/kg
Man	--[d]	--[d]	--[d]
(Fish)	NA[e]	NA	0.0006 mg/kg (2 days)
(Rabbits)	NA	NA	0.034 mg/kg (2 days)
(Deer)	NA	NA	0.043 mg/kg (2 days)
(Water)	NA	NA	0.0012 mg/kg (1 day)
(Berries)	NA	NA	0.0030 mg/kg (1 day)
(Mushrooms)	NA	NA	0.0015 mg/kg (1 day)
Toxicity Summary	LD_{50} >2,000 mg/kg in rabbits.	Nasal irritation in rats. No LC_{50} reported.	Acute oral LD_{50}'s ≥310 mg/kg for guinea pigs, rats, and rabbits.
	Most studies, LC_{50} >100 ppm for fish		

[a]Assumes aerial application at 3 lb active ingredient per acre and ground foliar application at 7.4 lb active ingredient per acre. See text for further explanation.

[b]Occupational doses include all routes of exposure.

[c]Fish exposure results from immersion in water containing triclopyr.

[d]Human environmental exposures via dermal and inhalation routes will be considerably less than occupational doses. Therefore, occupational doses are used as a worst-case rather than an estimate of environmental dermal and inhalation exposure. Oral environmental exposures are estimated for six sources.

[e]NA represents nonapplicable data.

Total occupational doses are, of course, a function not only of application rate and specific occupational category, but also of duration of exposure. Table 4-1 includes estimates of the number of days per year of exposure, by triclopyr application method and formulation used, in 1982. It should be noted, however, that in many instances, the same individuals are not exposed throughout the entire spray period.

4.2.2 Environmental Exposures

Environmental exposures in man occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with herbicide, or drink water containing herbicide. Animals, both terrestrial and aquatic, are subject to environmental exposures as well.

4.2.2.1 Dermal Exposures. Human dermal environmental exposures would be less than occupational exposures since only spray operators and observers are directly involved with actual activities on the spray units. A casual visitor to spray units should be expected to receive a dose higher and exposure much less by orders of magnitude than that of the observer receiving direct spray deposition. Animals in the target spray zone, however, are subject to dermal exposure. Rabbits and deer, representing both small and large game animals respectively, have the following estimated dermal exposures based on 3.0 lb a.i./acre aerial spray:

Rabbits in Aerial Spray Zone: 1.209 mg/kg (0.403 mg/kg at 1 lb/acre application rate)

Deer in Aerial Spray Zone: 0.303 mg/kg (0.101 mg/kg at 1 lb/acre application rate)

4.2.2.2 Inhalation Exposures. Human inhalation environmental exposures would be less than occupational exposures since spray operators, involved with activities on the spray units, are more likely to be subject to spray mist than is a casual visitor. Thus a casual forest visitor should be expected to receive an inhalation exposure orders of magnitude less than that of a backpack sprayer. Environmental inhalation exposures of animals in the spray target area would occur on a one-time basis and would be limited to a time frame that can be measured in minutes. Inhalation exposure is therefore expected to be so small that it can be neglected in this analysis.

4.2.2.3 Immersion Exposures. Aquatic organisms are subject to environmental exposures from immersion in streams and ponds adjacent to target spray zones. Assuming a buffer zone of approximately 100 feet between aerial spray zones and waterbodies, environmental exposures for aquatic organisms would be equivalent to the estimated concentration of triclopyr in water, calculated as:

Water concentration: 0.03 ppm (0.01 ppm at 1.0 lb/acre application rate)

This concentration of triclopyr represents the estimated maximum concentration immediately after spraying. Due to rapid water hydrolysis and rapid photodegradation, the persistance of triclopyr in water is expected to be very short with a half-life of less than 10 hours (Weed Science Society of America, 1983).

4.2.2.4 Oral Exposures. In terms of oral exposures, any incidental ingestion of triclopyr by workers on the spray unit would be accounted for by the estimates of occupational dose. Oral environmental exposures would occur for wildlife eating contaminated vegetation and for human consumption of fish, deer, rabbit, water, berries, and mushrooms. Basic assumptions for estimates of oral environmental exposures for both man and wildlife are presented in the General Introduction to the Herbicide Background Statements. Maximum reasonable oral exposures for deer and rabbits, representing game animals potentially eaten by humans, assume vegetation is sprayed from the ground at 7.4 lb a.i./acre and that rabbit browse is partially shielded by overstory. These estimates are as follows:

Deer: 10.656 mg/kg (1.44 mg/kg at 1 lb/acre application)
Rabbit: 7.770 mg/kg (1.05 mg/kg at 1 lb/acre application)

Estimates of human oral exposures from consumption of fish, deer, and rabbit require, as a starting point, estimates of maximum tissue concentrations of triclopyr in fish, deer, and rabbit.

Fish tissue concentrations are derived as follows:

- water concentrations: 0.03 ppm

- bioconcentration: 1.0 (studies with catfish and mosquito fish, summarized in Appendix F, indicate that triclopyr does not bioaccumulate in fish.)

Fish tissue concentration = 0.03 ppm (0.03 mg/kg)

Deer and rabbit tissue concentrations include triclopyr accumulated from both oral and dermal exposure. Tissue concentration estimates for deer and rabbit assume that dermally applied triclopyr is

absorbed at a rate of 10 percent as discussed in Section 4.2.1 with regard to spray observer absorption rates. As indicated in Appendix F, triclopyr administered intraveneously to various mammals was rapidly cleared, and excreted by the kidney. Given this rapid clearance and data indicating lack of biaccumulation in fish, it is assumed that only 10 percent of the ingested triclopyr will be assimilated by deer and rabbit. It is also assumed that oral exposure of deer and rabbit to triclopyr lasts only for 2 days due to the relatively short environmental persistence of triclopyr. Deer tissue concentrations of triclopyr are derived as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 0.303 mg/kg (dermal exposure) x 0.1 = 0.0303 mg/kg.

Oral Exposure contribution assumes 10 percent assimilation of two-days feeding, or 10.656 mg/kg/day (oral exposure) x 0.1 x 2 days = 2.1312 mg/kg.

Total Deer Tissue concentration = 0.0303 mg/kg + 2.1312 mg/kg = 2.1615 mg/kg (2.1615 ppm).

Rabbit tissue concentrations of triclopyr are derived in the same way as for deer, as follows:

Dermal Exposure contribution assumes 10 percent absorption of a single exposure, or 1.209 mg/kg (dermal exposure) x 0.1 = 0.1209 mg/kg

Oral Exposure contribution assumes 10 percent absorption of two-days feeding, or 7.770 mg/kg/day (oral exposure) x 0.1 x 2 days = 1.554 mg/kg.

Total Rabbit Tissue Concentration = 0.1209 mg/kg + 1.554 mg/kg = 1.6749 mg/kg (1.6749 ppm).

Based upon the above fish, deer, and rabbit tissue concentrations and human consumption rates, the following estimates of maximum human oral exposures to triclopyr from eating meat are derived:

Fish meat: 0.03 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0006 mg/kg

Deer meat: 2.1615 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.04323 mg/kg

Rabbit meat: 1.6749 mg/kg x 2 days x 0.5 kg/day/person ÷ 50 kg person = 0.0335 mg/kg

Water and plant consumption by man would result in the following oral exposures to triclopyr based on assumptions stated in the General Introduction to the Herbicide Background Statements at an application rate of 3.0 lb a.i./acre (aerial):

Water:	0.0012 mg/kg (0.0004 mg/kg at 1 lb/acre application)
Berries:	0.003 mg/kg (0.001 mg/kg at 1 lb/acre application)
Mushrooms:	0.0015 mg/kg (0.0005 mg/kg at 1 lb/acre application)

4.3 Hazard Assessment

Toxicological properties of troclopyr to man, fish, and small mammals have been summarized in Table 2-1 and detailed in Section 2 of this Herbicide Background Statement. Table 4-2 compares estimates for both occupational doses and environmental exposures in man and wildlife with reported toxicity information (dermal LD_{50} in rabbits, rat inhalation results, rabbit, rat and guinea pig acute oral $LD50$'s, and immersion LC_{50}'s for fish). The reported exposure levels at which toxic effects have been observed in experimental animals are considerably higher than levels estimated for Forest Service applications.

Small mammal studies have shown that triclopyr applied to the eye can be slightly to severely irritating and that triclopyr fed at rates up to 200 ppm in feed does not result in birth defects (in rats). Triclopyr is not mutagenic in bacterial and cytogenetic assays but is weakly positive in the rat dominant lethal assay. Triclopyr is not carcinogenic. These studies are summarized in Table 2-1 and detailed in the Appendices. Generally, they indicate that there should be no significant potential for adverse reproductive, mutagenic, or carcinogenic effects to individuals from triclopyr use, if proper care is taken during application.

Appendix A

Summary of Triclopyr Toxicity Data for Invertebrates and Microorganisms

Formulation	Organism	Nature of Exposure	Exposure Time	Effects[a]	Comments	References
Garlon® 3A	Shrimp (Crustacea)	Surface absorption	96 hours	LC_{50} = 895 ppm	—[b]	Dow Chemical Company data reported in Ghassemi et al. 1981 and in Weed Science Society of America 1983
Garlon® 3A	Crab (Crustacea)	Surface absorption	96 hours	LC_{50} >1,000 ppm	--	Dow Chemical Company data reported in Ghassemi et al. 1981 and in Weed Science Society of America 1983
Garlon® 3A	Oyster (Mollusca)	Surface absorption	48 hours	56 ppm < LC_{50} < 87 ppm	--	Dow Chemical Company data reported in Ghassemi et al. 1981 and in Weed Science Society of America 1983
Garlon® 3A	Lichen (Peltigera praetextata) and symbiotic algae, Nostoc sp.	Surface absorption	--	No significant effect on rate of nitrogen fixation by either organism at application rates typically used in forestry applications.	Results involved both laboratory and field studies.	Hellborn and Bergman 1977 in Ghassemi et al. 1981
Triclopyr (triethyl-amine salt)	Cladocera (Daphnia magna; Crustacea)	Surface absorption static test	48 hours	LC_{50} = 1,170 ppm (1,030 to 1,340 ppm)	No animals were killed at <336 ppm, all died at >2,000 ppm Water pH = 7.7 to 8.0, temperature = 6.7 to 68.5°F (19.6 to 20.3°C).	Gersich et al. (in press)
Triclopyr (triethyl-amine salt)	Cladoceran (Daphnia magna; Crustacea)	Surface absorption (Test and control solutions replaced 3 times a week)	21 days	LC_{50} = 1,140 ppm (950 to 1,590 ppm)	No observed mortality was 65%. Water pH was 7.8 to 8.1, temperature was 66.2 to 69.8°F (19 to 21°C).	Gersich et al. (in press)
Triclopyr	Soil microorganisms: Aerobacter aerogenes; Pseudomonas aeruginosa; Salmonella typhosa; Staphlococcus aureus; Aspergillus terreus; and Pullularia pullulamis	Surface absorption	72 hours	No apparent effect on growth of organisms at 500 ppm.	--	U.S. Environmental Protection Agency registration data reported in Ghassemi et al. 1981

[a]95 percent confidence limits in parentheses.
[b]Data not available or not applicable.

Appendix B

Summary of Triclopyr Toxicity Data for Fish

Formulation	Organism	Nature of Exposure	Time	Effects[a]	Comments	References
Triclopyr (butoxyethyl ester) Garlon®3A	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 0.74 ppm	—[b]	The Dow Chemical Company 1983a
	Bluegill			LC_{50} = 0.87 ppm		
Triclopyr (triethyl-amine salt) Garlon®3A	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 552 ppm	—	
	Bluegill			LC_{50} = 891 ppm	—	
Triclopyr (triethyl-amine salt)	Fathead minnow	Surface absorption, static bioassay	96 hours	LC_{50} = 245 ppm (224 to 269 ppm)	Average 0.22g fish, pH 7.7 to 8.2, temperature 62.6 to 63.7°F (17 to 17.6°C)	Mayes et al. (in press)
Triclopyr (triethyl-amine salt)	Fathead minnow	Surface absorption, flow-through test	96 hours	LC_{50} = 120 ppm (104 to 140 ppm)	Average 0.22g fish, pH 7.9 to 8.2, temperature 76.6 to 78.4°F (24.8 to 25.8°C).	Mayes et al. (in press)
			192 hours	LC_{50} = 101 ppm (88.5 to 116 ppm)		
Triclopyr (triethyl-amine salt)	Fathead minnow (embryo-larval stages	Surface absorption, flow-through test	31 days	At 114 ppm (measured), larval survival (2.1% at 28 days) significantly less than controls (79% at 28 days). No difference between treatment groups and controls in terms of means day-to-hatch (day 3); hatchability of embryos, normal larvae at hatch, and growth.	Fish treated at 0, 13, 20, 31, 48, 73 and 112 ppm nominal concentrations. Mean measured concentrations were: none detected, 11.7, 19.3, 29.2, 46.7, 72.7, and 114 ppm.	Mayes et al. (in press)
Triclopyr	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} = 117 ppm	—	The Dow Chemical Company 1983a
	Bluegill			LC_{50} = 148 ppm	—	
Garlon®3A	Rainbow trout	Surface absorption, static bioassay	96 hours	LC_{50} > 100 ppm	53.6°F (12°C), 0.9 gram fish	Johnson and Finley 1980
Garlon®3A	Bluegill			LC_{50} > 100 ppm	71.6°F (22°C), 0.8 gram fish	

[a] 95 percent confidence limits in parentheses.
[b] Data not available or not applicable.

Appendix C

Summary of Triclopyr Toxicity Data for Birds

Formulation	Organism	Nature of Exposure	Exposure Time	Effects	Comments	References
Triclopyr	Mallard duck	Acute oral	—[a]	LD_{50} = 1,698 mg/kg	—	The Dow Chemical data reported in Weed Science Society of America 1983
Garlon® 3A	Mallard duck	Acute oral	—	LD_{50} = 3,176 mg/kg	—	
Garlon® 4	Mallard duck	Acute oral	—	LD_{50} = 4,640 mg/kg	—	
Triclopyr	Mallard duck	Subchronic oral	8 days	LC_{50} > 5,000 ppm	—	The Dow Chemical Company 1983a
	Bobwhite quail			LC_{50} = 2,935 ppm	--	
	Japanese quail			LC_{50} = 3,278 ppm	--	
Triclopyr (triethylamine salt) Garlon® 3A	Mallard duck			LC_{50} > 10,000 ppm	--	
	Bobwhite quail			LC_{50} = 11,622 ppm	--	
Triclopyr (butoxyethyl ester) Garlon® 4	Mallard duck			LC_{50} > 10,000 ppm	--	
	Bobwhite quail			LC_{50} = 9,026 ppm	--	

[a]Data not available or not applicable.

Appendix D

Summary of Triclopyr Toxicity in Mammals

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Acute Oral Toxicity		
Triclopyr	Rat (male and female)	Details not specified	LD_{50} = 729 mg/kg (male) LD_{50} = 630 mg/kg (female)	—[a]	
Garlon® 4	Rat	Details not specified	LD_{50} = 2,000 to 2,500 mg/kg LD_{50} = 2,460 mg/kg (male) LD_{50} = 2,140 mg/kg (female)	--	The Dow Chemical Company 1983b and Weed Science Society of America 1983
Garlon® 3A	Rat	Details not specified	LD_{50} = 2,000 to 3,000 mg/kg LD_{50} = 2,830 mg/kg (male) LD_{50} = 2,140 mg/kg (female)	--	
Triclopyr	Rabbit, mixed	Details not specified	LD_{50} = 550 mg/kg	--	Weed Science Society of America 1983
Triclopyr	Guinea pig (male)	Details not specified	LD_{50} = 310 mg/kg	--	
			Acute Dermal Toxicity		
Triclopyr	Rabbit	Details not specified	LD_{50} > 2,000 mg/kg	--	
Garlon® 4	Rabbit	Details not specified	LD_{50} > 4,000 mg/kg	--	The Dow Chemical Company 1983b
Garlon® 3A	Rabbit	Details not specified	LD_{50} > 4,000 mg/kg	--	
			Primary Eye Irritation		
Triclopyr	Rabbit	Unspecified	Slightly irritating.	--	
Garlon® 4	Rabbit	Unspecified	Not an eye irritant	--	The Dow Chemical Company 1983b
Garlon® 3A	Rabbit	Unspecified	Severely injurious.	--	

Appendix D (Continued)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Inhalation Toxicity		
Triclopyr (unspecified)	Rat	1 hour exposure to 5.34 ppm	No effect.	--	Weed Science Society of America 1983
Garlon® 4	Rat	4 hour exposure to aerosol at a concentration of 0.82 ppm, particle size ≤ 10μ.	No deaths; caused only nasal irritation.	--	The Dow Chemical Company 1983b
			Subchronic Oral Toxicity		
Triclopyr	Rat (male and female)	Fed concentrations including 3, 10, 30 and 100 mg/kg/day in diet for 90 days.	In males, observed decreased body weight, decreased liver weight and increased relative kidney weight at highest dose of 100 mg/kg/day. No adverse effects in males at doses ≤30 mg/kg/day or in females at doses ≤100 mg/kg/day.	--	The Dow Chemical Company 1983b and Weed Science Society of America 1983
Triclopyr	Mouse (male and female)	Fed concentrations including 6, 20 and 60 mg/kg/day for 90 days.	In males, observed reduced liver weight at highest dose of 60 mg/kg/day and no adverse effects at 20 or 6 mg/kg/day. In females no adverse effects at doses ≤60 mg/kg/day.	--	The Dow Chemical Company 1983b
Triclopyr	Dog (beagle)	Fed doses of 5, 10 and 20 mg/kg/day in diet for 228 days and 2.5 mg/kg/day in diet for 183 days.	Doses of 5, 10 and 20 mg/kg/day for 228 days decreased weight gain and food consumption and produced liver and kidney effects ascribed to increased urinary retention of test material. Dose of 2.5 mg/kg/day for 183 days slightly reduced kidney excretion of the test material.	--	The Dow Chemical Company 1983b

Appendix D (Concluded)

Formulation	Organism	Nature of Exposure	Effects	Comments	References
			Subchronic Oral Toxicity (Concluded)		
Triclopyr	Monkey	20 and 30 mg/kg/day in diet for unspecified length of time.	No evidence of toxic effect. No effect on renal excretion.	--	The Dow Chemical Company 1983b
			Chronic Toxicity		
Triclopyr	Rat	Fed doses of 3, 10 or 30 mg/kg/day in diet for approximately 2 years.	No toxic effects. Not carcinogenic.	—	The Dow Chemical Company 1983b
Triclopyr	Mouse	Fed doses of 3, 10 or 30 mg/kg/day in diet for approximately 2 years.	No toxic effects. Not carcinogenic.	—	
			Reproductive and Teratogenic		
Triclopyr	Rat	Three generation feeding study at doses of 3, 10 or 30 mg/kg/day.	No adverse effects on reproduction, growth or maturation; not teratogenic.	--	The Dow Chemical Company 1983b and Hanley et al. in press
Triclopyr	Rat	Gavaged pregnant rats with 50 to 200 mg/kg/day on days 6-18 of gestation.	Mild fetotoxicity-delayed ossification of the skull occurred in offspring of pregnant rats receiving 200 mg/kg/day.	--	
Triclopyr	Rabbit	Gavaged pregnant rabbits with 10 or 20 mg/kg/day on days 6-18 of gestation.	Not teratogenic and not fetotoxic at 100 mg/kg/day.	--	
Triclopyr	Rabbit	Gavaged pregnant rabbits with 25, 50, or 100 mg/kg/day on days 6-18 of gestation.	Toxic to does at all doses. Increased mortality at all doses.	--	The Dow Chemical Company 1983b and Hanley et al. in press
			Carcinogenicity		
			See Chronic Toxicity studies above.		

[a]Data not available or not applicable.

Appendix E

Summary of Triclopyr Mutagenicity

Formulation	Organism	Nature of Exposure	Effects	Comments	References
Triclopyr	Not available	Bacterial assay systems (unspecified)	Nonmutagenic	—[a]	The Dow Chemical Company 1983b
Triclopyr	Not available	Cytogenetic assays (unspecified)	Nonmutagenic	—	
Triclopyr	Not available	Dominant lethal studies in rats and mice (unspecified)	Weakly positive in rats; negative in mice.	—	

[a]Data not available or not applicable.

Appendix F

Summary of Metabolism Studies with Triclopyr

Formulation	Organism	Dose	Comments	References
Triclopyr	Rat	5 mg/kg administered intravenously; 100 mg/kg administered intravenously	Urinary excretion accounted for 91% of dose. Urinary excretion accounted for 83% of dose.	The Dow Chemical Company 1983b
Triclopyr (^{14}C)	Rat	100 mg/kg (route of administration not clearly given, assumed to be oral)	Saturated blood plasma clearance curves.	
Triclopyr (^{14}C)	Dog	0.5 mg/kg (route of administration not given); 20 mg/kg (route of administration not given)	Half-life of 14 hours for clearance from blood plasma. Half-life of 96 hours for clearance from blood plasma.	
Triclopyr (^{14}C)	Monkey	Dose and route of administration not clearly given, assumed to be 30 mg or less/kg per day, repetitive daily doses.	Clearance from blood plasma occurred in a biphasic manner with one phase having a 3-hour half-life and one phase having a 151-hour half-life. 75.8% of the total ^{14}C activity was recovered in the urine, 95.7% of which was recovered within the first 24 hours.	The Dow Chemical Company 1983b
Triclopyr (^{14}C)	Rat	Dose not given, administered intravenously or orally.	Liver and fat tissues with tissue/plasma ratio >1 with both intravenous and oral administration.	
Triclopyr (^{14}C)	Dog	0.5 and 5.0 mg/kg administered intravenously	Kidney tissues with tissue/plasma ratio >1. Residues found only in the kidney.	
Triclopyr (^{14}C)	Dog	20 mg/kg administered intravenously	Kidney tissues with tissue/plasma ratio >1. Residues found in most other tissues, but with tissue/plasma ratio > 1.	The Dow Chemical Company 1983b
Triclopyr (^{14}C)	Dog	Dose not given, administered orally.	Kidney tissues with tissue/plasma ratio > 1.	
Triclopyr (^{14}C)	Monkey	30 mg/kg, administered intravenously	No tissues found with tissue/plasma ratio >1.	
Triclopyr	Catfish	Dose and route of administration not given.	Triclopyr and metabolites trichloropyridinol and trichloromethoxypyridine do not accumulate in fillets, heads, viscera, or skins.	Dow Chemical Company report cited in Ghassemi et al. 1981
Triclopyr	Mosquito fish	Dose and route of administration not given.	No significant bioaccumulation of trichlorpyridinol observed.	

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